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
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# DISTRIBUTION OF GLYCOGEN IN THE CENTRAL AND SYMPATHETIC NERVOUS SYSTEMS OF THE HORSE, WITH REFERENCE TO HISTOCHEMICAL ANALYSIS OF THE RELATION BETWEEN GLYCOGEN AND NISSL'S BODIES

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(With Plate I)

(Received April 19, 1937)

## INTRODUCTION

The distribution of glycogen in the central nervous system of vertebrates has been reported by many investigators. One of the most recent work is that of SATO (1930). This investigator found that the nerve cells in every centers of oxen, pigs, cats, rabbits, mice, dolphins, birds and reptiles contain more or less amount of glycogen, and even the cells of hypophysis, ependyme cells and cerebrospinal fluid contain a minute amount of it. TANAKA (1928-1929) studied the distribution of glycogen in the central nervous system of guinea-pigs, rats, hens and pigeons, and obtained results similar to that of SATO. KATASE and MITSUDA found that glycogen is contained in the cells of cortex cerebri, the PURKINJE's cells and spinal ganglion cells of dolphins, rats and mice. OHASHI (1922) studied the distribution of glycogen in the central nervous system of frogs (*Rana esculenta* and *Bufo bufo japonicus*) and stated that in summer glycogen appears only in both the chorioid plexus and the spinal cord, but in winter also in lobus opticus, cerebellum and ganglion cells. In spite of a vast literature as above mentioned, no one has studied the distribution of glycogen in the central and the sympathetic nervous system of the horse.

Since NISSL published his investigation of the nerve cells, the composition of the NISSL's bodies has been studied by many workers. MÜHLMANN (1912) investigated the solubility of the NISSL's bodies in various solutions, acids and alkalies, and stated that these bodies contain three different protein components. Later, this investigator (1929) showed that they contain nuclein compounds, because of the fact that they stain positively with FEULGEN's (1926) "Nuclealfärbung" and also is dissolved by



nucleases. \*These results were partially confirmed by EINARSON's (1932) staining experiment with dissolved lacks. On the other hand, UNNA and GANS (1914) denied that these bodies contain nuclein compounds, because they stain negatively with purified methyl green. EINARSON (1935) studied the influence of the varieties of the fixative and their pH upon the NISSL's pattern, together with the stainability with various stains and stated that NISSL's bodies contain at least three histological components: A, basophil chromatin substance; B, basophil protein; and C, acidophil protein. From these data one sees that there are discrepancies among authors concerning the histological or chemical composition of the NISSL's bodies, except that they contain some protein components.

TANAKA (1929) studied as to whether or not the NISSL's bodies are the same as the so-called glycogen granules appearing in the nerve cells and denied their similarity because of the fact that after the salivary digestion of the glycogen granules the NISSL's bodies appear by staining with toluidin blue. SATO (1930) stated that the ganglion cells of vertebrates, such as mammals, birds and amphibians, contain glycogen resembling the NISSL's bodies in shape. Thus no one has yet given reliable evidence for the existence of glycogen in the nerve cells, notwithstanding the general thought that the NISSL's bodies may contain glycogen or a substance related to it.

In the present investigation I have dealt with the distribution and the nature of glycogen in the central and the sympathetic nervous system of the horse, and histological analysis of the NISSL's bodies with especial reference to the relation of glycogen to the NISSL's bodies.

#### MATERIALS AND METHODS

All the material was taken from the horse. Three healthy animals of about 4 years old were selected. The jugular vein was cut without anesthesia and the animal was allowed to bleed to death. From the central nervous system, cerebrum, cerebellum, medulla oblongata, spinal cord, spinal ganglion, ganglion petrosum, ganglion Gasserii, ganglion jugulare, and from the sympathetic nervous system, ganglion trunci sympathici, AUERRACH's plexus and MEISSNER's plexus were obtained.

Alcohol-formalin saturated with sodium acetate (TORYU) or with magnesium sulphate (SATO) were used as fixing fluids. All the materials were embedded in celloidin and sectioned 20  $\mu$  thick.

The following stains were used. Methylene blue or toluidin blue was

used for NISSEL's bodies, BEST's carmin fluid for glycogen, and Sudan III for lipid.

The analyses of NISSEL's bodies were carried out by determining the affinity for various stains above mentioned after digestive treatment with various enzymes. The sections were put into the buffer solution of a known pH containing a known enzyme for a fixed time and then were used for staining. Amylolytic enzymes and proteolytic ones were commercial, but lipolytic one was obtained from the seeds of *Ricinus communis* (TAKAMIYA, 1935). The method was as follows: The dried powdered material without shell was mixed with 10 volumes of ether petroleum. After several hours the solution containing fat was filtered off. The residue was dried and mixed with 5 volumes of distilled water. After 18 hours the solution containing lipase was filtered. For the digestion of lipid, the enzyme solution was added in 5 per cent to the buffer solution of a moderate pH.

## RESULTS

### 1. Result Obtained for the Determination of Fixing Fluid.

For BEST's carmin staining of glycogen, different kinds of fixatives have been recommended by different workers: BEST, ARNOLD, NEUKIRCH, CARNOY, KINOSHITA, SATO, TANAKA, etc. None of them, however, are regarded as ideal for the glycogen in every tissue. Therefore, as the first step of this investigation, I have preliminarily tested the staining power of BEST's carmin fluid for glycogen in the nerve cells after fixation in several kinds of fixing fluids. As materials the spinal cord and the sympathetic ganglion of the horse were employed. All the material was embedded in celloidin and sectioned  $20\mu$  thick. Staining time was approximately 40 minutes. The results are given in Table 1. In the same table are also given the results obtained for the anterior lobe of the hypophysis and the liver as controls.

As will be seen in Table 1, chloroform combinations, such as CARNOY's fluid and alcohol-chloroform saturated with sodium acetate, prevented the carmin staining for glycogen in the nerve cells, although they really gave better results for the liver and the hypophysis. On the other hand, alcohol-formalin saturated with sodium acetate (TORYU) proved the best of all, showing perfect results in all respects. Therefore, this fixative was mostly employed throughout the entire course of this investigation.

TABLE 1.

*Stainability of glycogen in BEST's carmin fluid after fixation in various solutions.*

Fixing fluid	Reaction	Material				Shrinkage
		Spinal cord	Sympathetic ganglion	Anterior lobe of hypophysis	Liver	
Alcohol (BEST)	Neutral	+++	+++	+++	+++	+++
Alcohol-Ether	"	++	++	+++	+++	+++
Alcohol-Ether saturated with magnesium sulphate (KINOSHITA)	"	+++	+++	+++	+++	+
Alcohol-Ether saturated with ammonium sulphate (KINOSHITA)	"	+++	+++	+++	+++	+
Alcohol-Formalin (TANAKA)	"	+++	+++	++	++	+
Alcohol-Formalin saturated with magnesium sulphate (SATO)	"	+++	+++	+++	+++	+
Alcohol-Formalin saturated with ammonium sulphate (SATO)	"	+++	+++	+++	+++	+
Alcohol-Formalin saturated with sodium acetate (TORYU)	Alkaline	+++	+++	+++	+++	±
Formalin saturated with dextrose (NEUKIRCH)	Neutral	++	++	+	+	+++
Alcohol-Chloroform with glacial acet. acid (CARNOY)	Acid	+	+	+++	+++	±
Alcohol-Chloroform saturated with sodium acetate (TORYU)	"	+	+	+++	+++	-

## 2. Result Obtained for the Distribution of Glycogen.

The distribution of glycogen in both the central and the sympathetic nervous system was determined by the method already described, and the results are given in Tables 2 and 3.

As will be seen in Table 2, glycogen is contained in the nerve cells in every center. But remarkable variations are found in the amount of it among the cells. The cells of procencephalon and diencephalon contain a small amount of it, while those of mesencephalon, metencephalon, medulla oblongata, spinal cord, spinal ganglion and all the peripheral ganglia con-

TABLE 2.  
*Relative amount of glycogen in the nerve cells of the  
central nervous system.*

Regions		Glycogen	Regions		Glycogen
Proencephalon	Lobus frontalis	+	Mesen- cephalon	Pons	##
	Lobus parietalis	++		Cerebellum	+
	Lobus occipitalis	+		Nucleus dentatus	##
	Lobus temporalis	+		Oliva	##
	Cornu ammoni	++		Corpus restiforme	##
	Nucleus lentiformis	±		Cervical portion (Proximal)	##
	Nucleus claudatus	+		Cervical portion (Distal)	##
	Nucleus taeniaeformis	+		Thoracical portion	##
Diencephalon	Thalami optici	±	Spinal cord	Lumbar portion	##
	Glandula pinealis (in interstitial tissue)	##		Ganglion petrosum	##
	Hypophysis anterior	##		Ganglion Gasseri	##
	Hypophysis middle	++		Ganglion jugulare	##
Mesen- cephalon	Hypophysis posterior	—	Ganglion	Ganglion spinale	##
	Corpora quadrigemina	++			
	Crura cerebri	##			

TABLE 3.  
*Relative amount of glycogen in the sympathetic nerve cells.*

Ganglion	Glycogen	Ganglion	Glycogen
Ganglion cervicale craniale	##	Ganglion mesentericum cranialis	##
Ganglion cervicale caudale	##	Ganglion mesentericum caudalis	##
Ganglion thoracicale	##	Plexus Auerbachii	++
Ganglion lumbale	##	Plexus Meissneri	+
Ganglion sacrale	##		

tain a great amount of it, so that the whole body of the cells assumes an intense red color.

The nerve cells in the sympathetic ganglia contain a great amount of

glycogen, except those in the AUERBACH's and the MEISSNER's plexus in which only a small amount of it is observed.

Glycogen is also found in such cells, different from the nerve cells, as the cells of the anterior and the middle lobes of the hypophysis and also found in the interstitial tissue of the pineal body. But the nature of the glycogen contained in these cells and in the tissue just mentioned greatly differs from that contained in the nerve cells. This will be fully discussed under the section "Result Obtained for the Analysis of NISSL's Bodies."

### 3. Result Obtained for the Relation of Glycogen to the NISSL's Bodies.

I have preliminarily tested as to whether or not the fixing fluid for the glycogen in the nerve cells is applicable for the NISSL's bodies. Pieces of the spinal cord and the sympathetic ganglion were fixed in the same fixatives as those used for the glycogen. These materials were embedded in celloidin and stained with methylene blue or toluidin blue. The results are given in Table 4.

TABLE 4.  
*Stainability of NISSL's bodies with methylene blue  
after fixation in various solutions.*

Fixing fluid	Stainability	Fixing fluid	Stainability
Alcohol	##	Alcohol-Formalin saturated with magnesium sulphate	##
Alcohol-Ether	++	Alcohol-Formalin saturated with sodium acetate (TORYU)	##
Alcohol-Ether saturated with ammonium sulphate	##	Formalin saturated with dextrose	##
Alcohol-Formalin	##	Alcohol-Chloroform with glacial acetic acid (CARNOY)	##
Alcohol-Formalin saturated with ammonium sulphate	##	Alcohol-Chloroform saturated with sodium acetate	##

As is shown in Table 4, all the fixatives, except chloroform mixture, showed the same degrees of staining of NISSL's bodies as those of glycogen staining already mentioned. Thus excellent results were obtained from the materials fixed in alcohol-formalin saturated with sodium acetate (TORYU), which accordingly was mostly employed as fixing fluid for the NISSL's bodies.

#### A. Morphological Relation.

As the first step of investigations concerning the relation of glycogen within the nerve cells to the NISSL's bodies, the morphological relation between the two was investigated. The observations are as follows:

The form and the arrangement of glycogen resemble those of NISSL's bodies. Four types are found, showing a fairly constancy for the different varieties of nerve cells.

First type (*Motor cell type*). The typical form is found in the large motor cells in the anterior horn of the spinal cord, where the granules are spindle or roundish in form and of conspicuous size, which often exceeds in dimensions the large nucleolus of the cells. They are to be found not only in the cell body, but also for a distance in the dendrites, where they have a more elongated, spindle shape. Neither glycogen nor NISSL's bodies are found in the neurite and its cone of origin (Fig. 1). The cells in the most nuclei of crura cerebri, pons and medulla oblongata show this type, but the granules are by far smaller than those found in the anterior horn of the spinal cord.

Second type (*Sensory cell type*). The granules within the sensory nerve cells in the posterior horn of the spinal cord are typical. They are large, spindle shaped and very few in number. The cells in thalami optici, hippocampus, corpora quadrigemina, nucleus dentatus and those in some nuclei of crura cerebri and medulla oblongata also contain the granules of this type.

Third type (*Ganglion cell type*). In the cells of the spinal ganglia and of the peripheral ganglia they are roundish bodies of relatively large size and closely packed in the cell body. The cells in the sympathetic ganglia contain the granules of this type, except those in the MEISSNER's plexus and in the AUERBACH's plexus in which the granules are so small and so closely packed as to make the cell look dusty.

Fourth type (*Diffused type*). Neither glycogen nor NISSL's substance form granules in the cells of cortex cerebri and in PURKINJE's cells, where they are diffuse.

B. Stainability of NISSL's bodies with BEST's carmin fluid.

Glycogen staining was made on the sections preliminarily stained with methylene blue or with toluidin blue. The method is as follows:—

1. Decolorize the sections, preliminarily stained with methylene blue, with xylol, 96% alcohol, 80% alcohol.
2. Wash in water for few minutes.
3. Stain the sections for 30 minutes with DELAFIELD's haematoxylin diluted ten times.
4. Wash in water for few minutes.
5. Stain for 40 minutes in BEST's carmin fluid.
6. Differentiate in the mixture:—

Alcohol abs., 80 cc.

Methyl alcohol, 40 cc.

Aq. dest., 100 cc.

7. Wash off in 80%, 96% alcohol.

8. Creosot-xylol, balsam.

Stained by the method as above mentioned, red colored granules appeared and the form and arrangement of them were found to be strictly the same as those of the NISSL's bodies (Figs. 1 and 2). It was also found that the degree of the glycogen staining always shows a good agreement with that of the NISSL staining; namely, with the decrease in stainability with methylene blue of the NISSL's bodies, the stainability of the latter in BEST's carmin fluid decreases. Thus it was found that after disappearance of the NISSL's bodies by the action of digestive enzymes or by post-mortem autolysis, no glycogen granules was found by BEST's carmin staining (Figs. 3-6).

TANAKA (1929) studied as to whether or not the glycogen granules contained in the nerve cells of vertebrates are the same as the NISSL's bodies. He attempted to stain the NISSL's bodies with BEST's carmin fluid and failed to obtain any positive result. In the present investigation of the nerve cells of the horse, they could be stained with BEST's carmin fluid. This makes probable that the glycogen granules contained in the nerve cells are the same as the NISSL's bodies when the morphological relation alone is considered, or that the glycogen is contained in the nerve cells as one of the elements of the NISSL's bodies when not only the morphological relation, but also the componental relation are considered.

#### 4. *Result Obtained for the Histological Analysis of the NISSL's Bodies.*

To obtain further data concerning the evidence that the glycogen contained in the nerve cells is one of the elements of the NISSL's bodies, and also to determine whether or not some other elements are contained in the NISSL's bodies, further histological analysis with especial reference to the relation of glycogen for the NISSL's bodies was made. In this experiment digestive treatment and staining method were employed as follows:

Sections of  $20\mu$  thick were immersed in the buffer solution of a known pH containing a known enzyme, and at the fixed intervals they were used for the determination of the relation of glycogen to the NISSL's bodies and for the detection of other expected elements in the NISSL's bodies by staining with various stains already mentioned.

A. Relation of glycogen to the NISSL's bodies seen from the action of various enzymes.

Preceding the digestive treatment on the glycogen in the nerve cells, I have tested the digestive power of various enzymes on the glycogen in the liver cells and the His's bundle. The materials were obtained from the horse. The methods employed for the fixation and the embedding of the materials were the same as those of the nerve cells. The sections of  $20\mu$  thick were used. The results are given in Table 5.

TABLE 5.

*Digestive power of various enzymes on the glycogen in the liver cells and the His's bundle.*

Enzyme solution	pH	Time required for the complete digestion, in hours	
		Liver cells	His's bundle
Saliva (50%)	7	1-2	1-2
„ (25%)	7	1-2	1-2
„ (12.5%)	7	4	4
Diastase (1%)	6	4	4
Panctase (0.8%)	7	3-4	3-4
Trypsin (0.5%)	7	4	4
Pancreatin (2.5%)	7	4	4
Pepsin (1%)	5	Not digested	Not digested
Saliva (12.5%) Pepsin (1%)	6	4-5	4
Diastase (1%) Pepsin (1%)	6	4	4-5
Lipase solution	6-7	Not digested	Not digested

As will be seen in Table 5, any enzyme solution, as far as it contains amylolytic enzyme, digests the glycogen in the liver cells and the His's bundle, the time required for the complete digestion being about 4 hours in most enzyme solutions.

To determine whether or not the digestion of glycogen in the nerve cells occurs by the action of the same enzyme solution and also whether or not its digestion occurs at the same time when the NISSEL's bodies disappear, digestive treatment was made on the nerve cells. In this experiment each section was used for both the NISSEL staining and the glycogen staining by the method as already mentioned. The results are given in



Table 6. In the same table are also given the data obtained for the glycogen in the anterior lobe of the pituitary body and in the pineal body for comparison with that obtained for the glycogen in the nerve cells.

TABLE 6.  
*Digestive action of various enzymes on the glycogen and the NISSL's bodies in the nerve cells.*

Enzyme solution	Time required for the complete digestion of NISSL's bodies, in hours		Time required for the complete digestion of glycogen, in hours			
	Spinal cord	Sympathetic ganglion	Spinal cord	Sympathetic ganglion	Pituitary body	Pineal body
Saliva (50%)	10	10	10	10	1-2	1-2
" (25%)	13	15	13	15	1-2	1-2
" (12.5%)	15	20	15	20	4	4
Diastase	8-9	8-9	8-9	8-9	4	4
Pepsin	Not digested	Not digested	Not digested	Not digested	Not digested	Not digested
Pancreatic	3	3	3	3	4	4
Trypsin	4	4	4	4	4	4
Pancreatin	4	4	4	4	4	4
Pepsin saliva	8-9	8-9	8-9	8-9	4	4
Pepsin diastase	8-9	8-9	8-9	8-9	4	4
Lipase solution	Not digested	Not digested	Not digested	Not digested	Not digested	Not digested

As will be seen in Table 6, the NISSL's bodies were found to resist pepsin and saliva, to be slowly digested by diastase and easily by trypsin, pancreatic and pancreatin. The same relation was also found for the glycogen in the nerve cells. Furthermore, it was found that the degree of digestion of the glycogen was the same as that of the NISSL's bodies; namely the degree of glycogen staining at fixed intervals during the digestive treatment showed a good agreement with that of the NISSL staining (Figs. 3-6).

The glycogen in the pituitary body and the pineal body was easily digested by the action of saliva and diastase, the time required for the complete digestion being about 4 hours. This shows that the nature of glycogen in these tissues is the same as that of the glycogen in the liver

and in the His's bundle, but not the same as that of the glycogen in the nerve cells.

TANAKA (1928-1929) stated that the glycogen in the nerve cells of vertebrates was digested by the action of saliva and diastase. SATO (1930) also found that the digestion was completed within 6 hours, being more rapid than the digestion of the glycogen in the liver cells. From the results obtained for the digestion of the glycogen in the nerve cells of the horse by the present author, however, it is noted that trypsin, pancreatin and pancreatin are digesting enzymes, but saliva and diastase and those with pepsin are not.

MACALLUM (1898) and SCOTT (1899) found the Nissel's bodies resist peptic digestion, and are slowly digested by trypsin. This was partially confirmed by the present investigation of the nerve cells of the horse.

At any rate, the digestion of both the Nissel's bodies and the glycogen granules in the nerve cells was completed in the mixture of three kinds of enzymes, proteolytic, amylolytic and lipolytic enzyme, suggesting that the Nissel's bodies at least contain three kinds of components, protein, glycogen and fatty substance. It seems to me that among these glycogen and protein are firmly bound with each other. To support the view just stated I noticed the following relations; first, the digestion of glycogen was completed by the same enzymes as those for the Nissel's bodies; second, after the disappearance of the Nissel's bodies by a digestive treatment or by a post-mortem autolysis no glycogen granules were found; third, the Nissel's bodies were able to stain with BEST's carmin fluid by the method as already mentioned.

B. Result obtained for the presence of lipid in the Nissel's bodies.

In the previous section it has already been suggested that the Nissel's bodies or the glycogen granules may contain fatty substance from the fact that they are only digestible by enzymes which act as digesting enzymes for protein, carbohydrate and fat. To obtain decisive evidences for the presence of the substance in these bodies or granules the following investigation was made.

a. Action of lipase on Nissel's bodies.

As the first step of investigations concerning the reliable evidences for the presence of lipid, the action of lipase for the Nissel's bodies or the glycogen granules in the nerve cells was determined. The method was as follows: 5 parts of lipase solution obtained from the seeds of *Ricinus communis* by the method already mentioned were added to 95 parts of a known enzyme solution which does not act as a digesting enzyme for

the NISSL's bodies. Sections from the materials fixed in alcohol-formalin saturated with sodium acetate were put into the solution just mentioned and after the fixed time the sections were used for the NISSL staining

TABLE 7.

*Digestive power of various enzyme solutions containing lipase for the NISSL's bodies and the glycogen granules.*

Enzyme solution	pH	Time required for the complete digestion of NISSL's bodies and of glycogen in hours	
		Spinal cord	Sympathetic ganglion
Saliva (25%) Lipase solution	6-7	8-10	8-10
Saliva (12.5%) Lipase solution	6-7	8-12	10-12
Diastase (1%) Lipase solution	6	3	3
Pepsin (1%) Lipase solution	6	5-6	5-6
Saliva (12.5%) Pepsin (1%) Lipase solution	6	4	4
Diastase (1%) Pepsin (1%) Lipase solution	6	3-4	4
Lipase solution	6	Not digested	Not digested

TABLE 8.

*Digestive power of various enzyme solutions containing no lipase for the NISSL's bodies and the glycogen granules in the nerve cells preliminarily treated with lipase.*

Enzyme	pH	Time required for the complete digestion of NISSL's bodies and glycogen, in hours	
		Spinal cord	Sympathetic ganglion
Saliva (25%)	7	8	8
Saliva (12.5%)	7	8	8
Diastase (1%)	6	2-3	3
Pepsin (1%)	6	3	4
Saliva (12.5%) Pepsin (1%)	6	3	4
Saliva (12.5%) Diastase (1%)	7	3	3
Diastase (1%) Pepsin (1%)	6	3	3

and the glycogen staining. The following method was also employed: Sections were put into the buffer solution containing lipase alone for 1 hour at 38°C. and then were subjected to digestion in various enzyme solution containing no lipase. The results are given in Tables 7 and 8.

It was found in the previous section that the NISSEL's bodies and the glycogen in the nerve cells were slowly digested by diastase, but not completely by saliva and not at all by pepsin. As will be seen in Table 7, however, pepsin and diastase to each of which was added lipase were found to be greatly active; namely the digestion of the NISSEL's bodies and of the glycogen was completed within from 3 to 4 hours in the solution containing diastase and lipase, from 5 to 6 hours in that containing pepsin and lipase and from 8 to 12 hours in saliva containing lipase. It seems to me that since commercial diastase usually contains a trace of proteolytic enzyme, and pepsin a trace of amylolytic enzyme, they were made active by the addition of lipase so that the digestion of the NISSEL's bodies and the glycogen granules was easily performed, but since saliva contains almost no proteolytic enzyme, the digestive action of its mixture with lipase is very weak.

As is shown in Table 8, the NISSEL's bodies and the glycogen granules in the sections which were put into the lipase solution for 1 hour before use were easily digested by diastase, pepsin and these combined with lipase, and slowly digested by saliva alone, showing that the fatty substance contained in the NISSEL's bodies was decomposed by the action of lipase.

At any rate, from the results above described it is noted that the NISSEL's bodies contain a fatty substance digestible by the action of lipase, besides protein and glycogen. The fatty substance in question just stated should be lipid, insoluble in alcohol and ether, because the sections used in this digestive treatment were free from fat, the latter being dissolved in alcohol and in ether during preparation.

b. Action of lipase on NISSEL's bodies fixed in CARNOY's fluid.

To determine whether the lipid contained in the NISSEL's bodies is soluble in chloroform or not the sections from the materials fixed in chloroform mixtures, such as CARNOY's solution and that saturated with sodium acetate, were put into the various enzyme solutions containing no lipase, and after a fixed time the digestive power of these enzyme solutions for the NISSEL's bodies and the glycogen granules was determined by the staining method already mentioned. The results are given in Table 9.

The following treatment was also made for the same purpose: The sections from the materials fixed in ordinary solution (TORYU) were put into pure chloroform and incubated at 38°C. for 24 hours. After washing well in water, they were used for digestion in the same enzyme solutions as the above. The results are given in Table 10.

TABLE 9.

*Digestive power of various enzymes for the NISSI's bodies and the glycogen in the nerve cells fixed in CARNOY's solution.*

Enzyme	pH	Time required for the complete digestion of Nissi's bodies and glycogen, in hours		
		Spinal cord	Jugular ganglion	Sympathetic ganglion
Saliva (25%)	7	8-10	8-10	8-12
Saliva (12.5%)	7	6-10	8-10	8-10
Diastase (1%)	6	3	4	4
Pepsin (1%)	5-6	4	4	4
Saliva (12.5%)	6	3	3	3
Pepsin (1%)	6	3	3	3
Saliva (12.5%)	7	3	3	3
Diastase (1%)	6	3	3	3
Diastase (1%)	6	3	3	3
Pepsin (1%)	6	3	3	3

TABLE 10.

*Digestive power of various enzymes for the NISSI's bodies and the glycogen granules after treatment with chloroform.*

Enzyme fluid	pH	Time required for the complete digestion of Nissi's bodies and glycogen, in hours		
		Spinal cord	Jugular ganglion	Sympathetic ganglion
Saliva (25%)	7	8-10	8-12	8-10
Saliva (12.5%)	7	8-12	8-12	8-10
Diastase (1%)	6	3-4	5	5
Pepsin (1%)	5-6	4-5	5-6	5-6
Saliva (12.5%)	7	3	4	3
Diastase (1%)	6	4	4	4
Saliva (12.5%)	6	4	4	4
Pepsin (1%)	6	3	4	4
Diastase (1%)	6	3	4	4
Pepsin (1%)	6	3	4	4

As has already been mentioned, diastase, pepsin, saliva and their mixtures do not act as digesting enzymes for the NISSL's bodies and the glycogen within the nerve cells fixed in alcohol-formalin saturated with sodium acetate or with magnesium sulphate. As will be seen in Table 9, however, these act as digesting enzymes for the NISSL's bodies and the glycogen granules in the nerve cells fixed in CARNOY's solution, the time required for complete digestion being about 4 hours in 1 per cent solution of diastase and of pepsin, about 3 hours in 12.5 per cent saliva mixed with 1 per cent solution of pepsin or of diastase and 8-10 hours in saliva containing no other enzymes. As will be seen in Table 10, almost similar results are also obtained from the ordinary sections treated with chloroform before use. These results show that the lipid contained in the NISSL's bodies was dissolved in chloroform, and accordingly the digestion of these bodies was completed without lipase.

KINOSHITA (1928) and SATO (1930) stated that after fixation in CARNOY's fluid the glycogen granules in the nerve cells were weakly stained with BEST's carmin, agreeing with the result obtained for the nerve cells of the horse by the present author as has already been stated in the section "Result Obtained for the Determination of Fixatives". TANAKA (1929) also stated that the glycogen in the nerve cells of vertebrates fixed in CARNOY's solution was almost unstained with BEST's carmin. Such disadvantage of glycogen staining seem to be related with the lack of lipid in the NISSL's bodies. To support the view just stated, I noticed that when these bodies contained lipid an excellent result of glycogen staining was also obtained as has already been found in the materials fixed in the solution containing no chloroform, such as alcohol-formalin saturated with sodium acetate (TORYU) or with magnesium sulphate (SATO).

c. Stain for lipid.

The presence of lipid in the NISSL's bodies was also determined by staining methods. Celloidin sections or frozen sections were obtained from the materials fixed in various solution and stained in a saturated solution of Sudan III in 65 per cent alcohol for 24 hours or longer, or in a saturated aqueous solution of Nile-blue sulphate for 12 hours.

The nerve cells fixed in alcohol-formalin saturated with salts, such as sodium acetate and magnesium sulphate, and stained with Sudan III are filled with small lipid granules, appearing deep brown. The form of these granules is almost the same in different varieties of nerve cells, and therefore no morphological relation of them to the NISSL's bodies is found. But lipid is undoubtedly one of the NISSL's bodies from the fact that

the digestion of these bodies was not completed, unless lipase is present as has been already stated.

A reaction of Nile-blue sulphate for lipoid is also positive. In this case the lipoid granules appear bluish red.

The nerve cells from the materials fixed in CARNOY's solution and the cells, fixed in ordinary solution and digested by lipase, are negatively stained with the stains above mentioned, showing that the lipoid contained in the NISSL's bodies was dissolved in chloroform or decomposed by lipase.

Thus it is noted that the NISSL's bodies contain lipoid, insoluble in alcohol and ether, soluble in chloroform and digestible by lipase. It is considered, however, that the lipoid is not necessarily essential to the NISSL's bodies, because after the lipoid was dissolved in chloroform or decomposed by the action of lipase the NISSL's bodies were found to be deeply stained with methylene blue or with BEST's carmin fluid as has already been stated.

#### SUMMARY

The results obtained in this investigation may be summarized as follows :

1. The nerve cells of procencephalon and diencephalon contain a small amount of glycogen, while those of mesencephalon, metencephalon, medulla oblongata, spinal cord and every peripheral ganglion contain a large amount of it.

2. The nerve cells in the sympathetic ganglia contain a great amount of glycogen, while those in AUERBACH's and MEISSNER's plexus contain but a very small amount of it.

3. The form of the glycogen granules is quite constant for the different varieties of nerve cells. Four types are found : A, motor cell type ; B, sensory cell type ; C, ganglion cell type ; and D, Diffused type.

4. Glycogen granules are to be found not only in the cell body, but also for a distance in the dendrites. The only parts of the cell that are free from them are the neurite and its cone of origin.

5. For the digestion of glycogen in the nerve cells a harmonious cooperation of amylolytic, proteolytic and lipolytic enzyme was necessary. It was not completely digested by the action of saliva or diastase alone.

6. Glycogen in the nerve cells is contained in the NISSL's bodies. The evidences are as follows : first, the NISSL's bodies were found to stain with BEST's carmin fluid by the method of the present author, as far as the NISSL staining is possible the glycogen granules of the same form

and arrangement as the NISSL's bodies appearing; second, when the NISSL's bodies were digested by enzymes no glycogen was found.

7. The NISSL's bodies also contain lipid. But the element is not essential to the NISSL's bodies, because after it was dissolved in chloroform or digested by lipase the NISSL's bodies were still found.

8. The interstitial tissue of pineal body and the cells of anterior and middle lobes of pituitary body contain a great amount of glycogen. The nature of this glycogen is the same as that of the glycogen in the liver and HIS's bundle. It was easily digested by saliva and diastase.

9. It was found that CARNOY's solution is not applicable for the fixation of the NISSL's bodies and the glycogen granules in the nerve cells; namely the lipid contained in the NISSL's bodies was dissolved in the solution, and also the glycogen granules weakly stained with BEST's carmin fluid.

10. On the contrary, however, alcohol-formalin saturated with sodium acetate (TORYU) gave better results than the known fixatives; namely, all the elements of the NISSL's bodies were perfectly protected and therefore these bodies were deeply stained with methylene blue and with BEST's carmin fluid.

Before leaving the subject, I wish to express my hearty thanks to DRs. S. HATAI and S. NOMURA for their valuable suggestions and criticism throughout the entire course of this work. My thanks are also due to DRs. H. IWATA, K. KIKUCHI and H. TUGE who helped me with much kindness during this work and to Mr. K. CHIBA who helped me to take materials.

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#### EXPLANATION OF PLATE I

- Fig. 1. Nissl's bodies in the motor cell of the spinal cord.  $\times 650$ . (No Nissl's bodies are seen in the neurite and its cone of origin.)
- Fig. 2. Glycogen in the same cell as the above. (The Nissl's bodies were decolorized and then stained with Best's carmin fluid.)  $\times 650$ . (The form and arrangement of glycogen granules are the same as those of the Nissl's bodies. The neurite and its cone of origin also contain no glycogen.)
- Fig. 3. Nissl's bodies in the motor cell of the spinal cord after treatment with 2.5 per cent. solution of pancreatin for 2 hours.  $\times 650$ . (Nissl's bodies are half digested.)
- Fig. 4. Glycogen in the same cell as the above.  $\times 650$ . (Glycogen is also half digested.)
- Fig. 5. Nissl's bodies in the motor cell of the spinal cord after digestion in 2.5 per cent solution of pancreatin for 4 hours.  $\times 650$ . (Nissl's bodies are completely digested.)
- Fig. 6. Glycogen in the same cell as the above.  $\times 650$ . (Glycogen is also completely digested.)



Fig. 1



Fig. 2

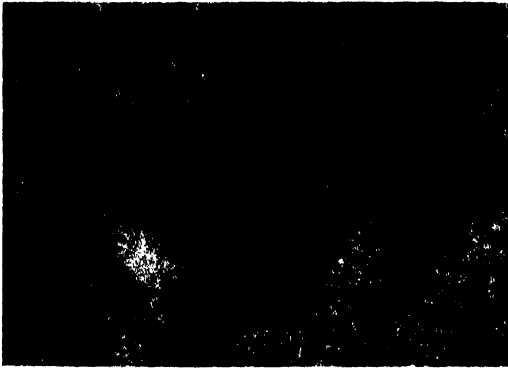


Fig. 3.

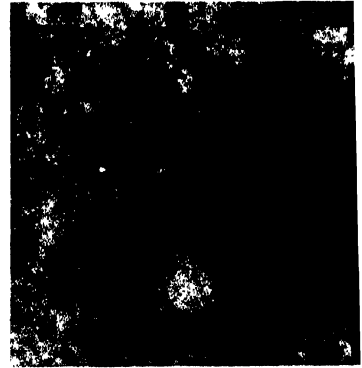


Fig. 4.

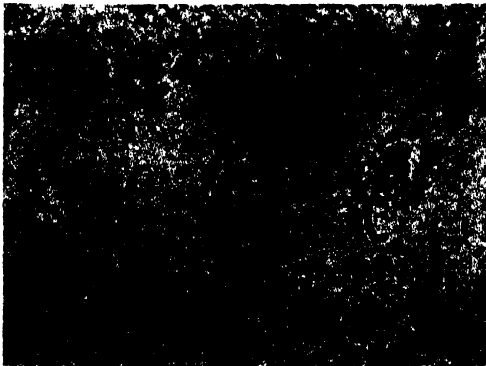


Fig. 5.

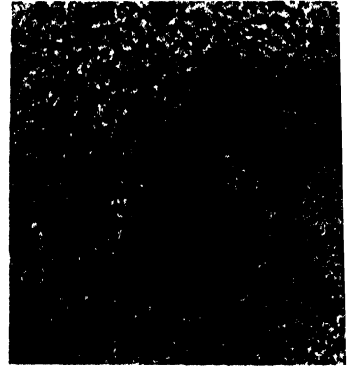


Fig. 6.



# THE REACTIONS OF THE MELANOPHORES OF EMBRYONIC AND LARVAL SALMON *ONCORHYNCHUS KETA*<sup>1)</sup>

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## INTRODUCTION

The problem of mechanisms in the chromatophores of teleosts has been studied by a vast number of investigators either to find out the biological significance of color change, or because of neurological interest in the subject. Most of the investigators have dealt with adult forms alone. So there is a great lack of literature on the mechanisms of the chromatophore change in developing fish in general. In view of the present status of our knowledge, therefore, it is essential, in order to secure a proper understanding and interpretation of the results obtained from

<sup>1)</sup>Aided in part by a grant from the Japan Society for the Promotion of Scientific Research.

the investigation of adult forms on which our present knowledge is exclusively based, to study the nature of the chromatophores in embryonic and larval fish.

It is generally accepted that, as first reported by BABÁK ('10) who worked with Mexican axolotl, the response in the melanophores of larvae is different from that which occurs in the adult in that in their very early stages their melanophore response depends exclusively upon the direct stimulation of their own cells, but in their later stages the reaction comes from indirect stimulation through the nervous system induced by impulses arising from the stimulation of the retinae. BABÁK refers this to the lack of the development of the eyes, or of the pigment controlling function of the retinae, in very young larvae. (See also LAURENS, '15, p. 592). He finds that in a very early stage the larvae are pale in complete darkness, but dark in bright light, while however in a later stage the reactions are quite the reverse, i. e., they are dark in complete darkness, and in bright light either pale or dark depending upon their backgrounds. Such melanophore reactions brought about in the early stages are called the primary phase and those in the later stages the secondary phase. The view above outlined was greatly expanded by LAURENS ('15) in his work on *Amblystoma* larvae and by FISCHER ('20) in his investigations of various amphibians.

Recent investigation by DUSPIVA ('31), working upon the two fish, *Salmo salvelinus* and *Perca fluviatilis*, has confirmed this to be true in the embryonic and larval fish. More recently TOMITA ('36), also working upon a number of fish larvae, reported results similar to that of DUSPIVA. Such a conception, however, does not appear to hold for all the members of fish larvae since studied (cf. Trout by LOWE, '17; *Fundulus* by WYMAN, '24a and GILSON, '26; *Cregonus fera* and two species of *Salmo* by BECHER, '29). Among these investigators, BECHER reports that in the fish larvae on which he worked the earliest melanophore response observed was dependent upon the different environments to which the larvae were subjected, omitting thus the so-called primary phase in their melanophore reactions. The dogfish, an ovoviviparous fish, according to the investigations of PARKER ('36), yields a similar result to that found by BECHER, there possibly occurring in the earliest stage a reaction identical with that in the adult, although this finding is contrary to that made by TOMITA who worked with the three ovoviviparous tropical fish, *Lebistes*, *Gambusia*, and *Xiphophorus*.

Special studies as to the effect of various drugs on the melanophores

of the developing fish have only occasionally been referred to in literature, but, in so far as the writer is aware, the papers of LOWE ('17), WYMAN ('24 a) and DUSPIVA ('31) are the only ones to merit special attention. The attempt to find out what effect chemicals, notably alkaloids, have upon the larval melanophores in which development is taking place, just as many previous workers have discovered such effects upon adult forms, should not be without significance.

In view of the lack of adequate knowledge as to the embryonic development of melanophores, the experiments here presented were undertaken in hopes of finding out whether any relation existed between the activities of both the visual and melanophoral systems in development, and of attempting, if possible, an analysis of the relation discovered. The present investigation was planned with a view to determining the reactions of the embryonic and larval melanophores by studying those normally developing, those in which the eyes and nervous system had been destroyed, and those treated with various chemicals, while exposing them to the conditions of light, darkness, and different backgrounds. It need also be said that the fish on which the writer experimented hatched out with a considerably smaller number of melanophores and developed much more slowly than the other fishes studied by previous writers referred to, so that he had the advantage of observing them very thoroughly and accurately.

#### MATERIAL AND METHODS

In the experiments herein described the salmon, *Oncorhynchus keta*, was used<sup>2)</sup>. The observations were made upon the eggs and larvae of the salmon caught in the river Abukuma near Sendai during the months of October, November and December of 1935, and in December of 1936. As soon as possible the eggs of the salmon caught from the river were artificially fertilized at the hatchery situated near the river. After this treatment the fertilized eggs were brought into the laboratory and allowed to develop in the specially constructed hatchery box through which running water was circulated. Other eggs too, which had developed to a certain degree at the hatchery, were brought in for use.

For detecting the time of the first appearance of the melanin granules the developing eggs were examined under a binocular microscope from time to time after having been cut out of the shell.

<sup>2)</sup>In this paper the word 'embryo' refers to a developing fish which lives in the egg shell, but the word 'larva' to the stage from the time of hatching to the absorption of the yolk.

When used for experiments the embryos and larvae were maintained in glass vessels of different sizes, the outside of which had been painted either white or black. These vessels were placed, when required, in a box, one side of which was left open so that the interior could be illuminated by an electric lamp, Mazda 100 watts, from a height of about 90 cm. Other details of the experimentation will be described separately under each heading.

#### DEVELOPMENT OF EMBRYONIC AND LARVAL MELANOPHORES

The development and distribution of the melanophores in this embryo are of interest since the melanin formation comes only in a very late embryonic stage in contrast with that of many other teleosts. After fertilization the embryo lives more than two months in the egg shell in the embryonic stage. Although the embryo in the shell cannot be clearly seen from without, melanin granules begin to appear in the head region about 10 days before hatching. Thus the time of the first appearance of melanin granules can be said to be nearly two months after fertilization, although this is dependent upon the temperature of the water. For example, a number of eggs, obtained by stripping from a female, were artificially fertilized on November 19, 1935, and were allowed to develop in the laboratory in a developing box with running water. The larvae in this lot began to hatch between February 10 and 20, 1936, at a temperature of 10° to 4°C. While developing the egg shells were explored from time to time, and it was found that a very small number of melanin granules could at first be seen in the region of the head on February 3, lying mostly over the brain, and were found scattered also in the region of the neck on February 8. The embryos of this age could be kept alive in a dish after removal from the egg shell.

At the time of hatching the larvae measure from 18 to 20 mm. in body length, the caudal fin excluded. Usually they bear a number of melanophores distributed in the head region, while a smaller number are found greatly diffused over the trunk and caudal regions, and in a slightly advanced form a still smaller number are seen in the caudal fin, but none are present in the yolk sac. At this advanced stage they may be divided into two types: one a very large type lying over the brain, the other a smaller type distributed within the trunk region, as noted by GILSON ('26) in his work on *Fundulus* embryo. The melanophores in this fish appear to develop in a cephalocaudal progression. Accordingly in this specimen

it is possible to trace and study the development of the melanophores after its larval stage has set in.

At the time of hatching the melanophores are all slightly expanded normally. As time goes on, that is, within 10 days after hatching, the melanophores spread more and more over the whole body including the caudal fin.

In the present specimens it takes about 50 days from the time of hatching to the absorption of the yolk sac. For the sake of convenience, the relation between the body length and the days after hatching are shown in the following table, though since the temperature of the water was not regulated in the present experiments,—it ranged from 5° to 12°C.,—we will merely indicate the ages of the larvae which are dealt with in the text.

TABLE 1.

*Showing the relation between the body length and the days after hatching (Oncorhynchus keta).*

Days after hatching	Hatching	5 days	10 days	20 days	30 days	40 days	50 days (yolk almost absorbed)
Body length (mm.)	19—20	21—22	22.5—23	26—26.5	27.5—28	29 30	31—32

The above data were obtained from the larvae hatched on April 1, 1936. The temperature of the water ranged from 8° to 12°C. In this batch there were found several larvae the yolks of which were completely absorbed by May 12, 1936.

#### REACTIONS OF THE MELANOPHORES TO CHANGES IN ENVIRONMENT

In studying the melanophore reaction of young fish it is of prime importance to determine whether or not the larvae react in the same manner as the adult to changes in environment, or rather, whether the young melanophores function from the beginning in adapting themselves to their environment. It is known that in the case of a certain fish two phases are present in melanophore reaction in the larval stage. (These phases are described in the above introductory note).

Now the larvae experimented upon immediately after hatching were placed in groups of ten in a vessel, some in a completely dark room, some in an illuminated black-walled vessel, and some in an illuminated white vessel respectively, and were left there during the time necessary for observation. Microscopic observations were made once or twice a



day. Care was taken to inspect the state of the melanophores as quickly as possible before their shape could change.

Some of those which were placed in complete darkness from the time of hatching tended to have slightly expanded melanophores, continuing thus in a slightly expanded condition for several days, but others did not show any change from the condition in which they were when they were first taken from the trough (p. 39). Daily inspection of those which remained in complete darkness showed however that this slightly expanded condition lasted for nearly three weeks. When they had attained a length of about 27 mm. (see Table 1), the larvae became lighter in color. As development advanced, they became extremely pale and almost all of the melanophores became concentrated. Such a condition persisted until after the yolk was completely absorbed. Such experiments were made upon a number of larvae taken from different batches, but with similar results.

A similar test, which yielded the same results, was made in which the larvae of an early stage, whose melanophores had been contracted by subjection to light, were put in complete darkness. It was found that the melanophores returned to a slightly expanded condition, which is always the normal condition of the larvae at about this stage. As development advanced, the melanophores of those which were still in complete darkness became gradually contracted. We found that such a transition from an expanded to a contracted condition, when we measured a number of specimens similarly treated, occurred about 20 days after the time of hatching, the body length at the time being about 27 mm., which accords with the table given above. If older larvae, either pale or dark in color, with a body length of more than 27 mm., which had not been placed in complete darkness, were now placed in complete darkness, they remained in, or turned to a pale condition, irrespective of their previous tints. Such color changes in the older larvae generally occurred between one and two days after they had been placed in complete darkness. Thus the change to a slightly expanded condition in complete darkness which the larvae undergo which are less than 20 days old and pale in color should not necessarily be attributed to the stimulation of darkness, but rather to the fact that these young larvae had not been subjected to illumination when they were placed in complete darkness, in which a slightly expanded condition would be normal.

When newly hatched larvae were placed in an illuminated black-walled vessel, their melanophores did not undergo any definite changes for several days. In young larvae from 10 to 15 days old the melanophores in the

tail region and the caudal fin appeared to be somewhat contracted, but most of those in the head remained slightly expanded. In the region of the trunk, however, no definite change was observed. In larvae which were more than about three weeks old the melanophores of the whole body became expanded. This occurred several hours after they had been placed in the vessel.

Again, larvae of different ages were put in an illuminated white-walled vessel. Although the time of response to such an environment was not definitely determined, the melanophores became contracted within several hours. Even at the time of hatching there was indication that a concentration of the melanophores was taking place. Embryos about to be hatched, in which a few melanophore cells were formed, were taken out of the egg shell and treated in the same way as the above. The reaction of the embryonic melanophores to light was essentially the same as that of the melanophores of the larval stage, in that the melanophores became concentrated in a few hours.

In so far as the above observation goes, the melanophore reactions to color environment indicate that in their very early stages the larvae do not react to darkness, while in the later stages they actively contract their melanophores.

#### EXPERIMENTS UPON BLINDED FISH

One or both eyes of the larvae at the time of hatching were removed by means of electro-cautery under chloretone anesthesia. The eyeless larvae were examined to ascertain whether or not any color changes would take place under varying environment. They were compared from time to time with larvae intact, both of which were placed in the same vessel. The vessels used were illuminated, black-walled and white-walled. Other larvae were placed in complete darkness and in dim light. During the first day after the operation there was no difference between the blinded and the unimpaired larvae that was recognizable. From the following day on, however, the blinded fish were found to have much more greatly expanded melanophores than those that were intact, the latter of which had been treated normally in dim diffused light. As the melanophores in the body surface increase in number, the tint assumed in the blinded larvae became a deep black, so that they were easily distinguishable from the unimpaired larvae.

Certain of the blinded larvae were placed in illuminated, white-walled

and black-walled vessels. The dark tint induced by enucleation remained unchanged in an illuminated vessel with white walls as well as in that with black walls, if the larvae were, say, less than 20 to 25 days old. This was the case also when they were placed in complete darkness. As for older larvae with enucleated eyes, there was not sufficient material to allow this test to be made, because death occurred generally within 20 days after operation. However, in the case of one 30-day larva from which both eyes had been removed 30 days previously and which continued to live, no apparent change was observed when it was placed in an illuminated black-walled vessel. After the above test was finished, the same specimen was placed in an illuminated white-walled vessel for 48 hours with the result that there seemed to be a slight tendency to paling. When it was placed in complete darkness, there seemed to be a few more contracted melanophores in comparison with larvae under normal conditions, although the fish was still invariably dark in color.

The one-eyed younger larvae presented a somewhat irregular picture of the melanophores, some of them expanding, others remaining unchanged, but that they were darker in tint than those intact of the same age was not apparent. As development advanced, however, they seemed to assume under a dim diffused light a tint of a little darker shade than the control. When they were placed in an illuminated white-walled vessel, the melanophores tended to become concentrated possibly to a smaller degree than the normal larvae in the same vessel. However, in cases where they were placed in an illuminated black-walled vessel, the larvae with one eye behaved quite similarly to those that were normal.

#### EXPERIMENTS UPON LARVAE WITH A DENERVATED AREA OF THE FIN

WYMAN ('24) found that in *Fundulus*, if a small cut were made transverse to the rays of the caudal fin, the cut gave rise to a dark band, extending from the cut to the posterior end of the fin. This method of studying the mechanism of melanophore changes has been used since by a number of investigators who have worked upon adult fish, but much less attention has been paid to its application to larval forms.

In the present experiments, operation under chloretone anesthesia was made upon the caudal fin of older larvae by means of a small pointed knife. In this way a denervated area was obtained in which there was very little interference with the blood circulation. After recovery from the anesthesia, that is, from one to two hours later, the melanophore

cells in the denervated area were found to be very widely dispersed, causing the so-called dark band to appear. In spite of its very wide dispersion each color cell seemed to assume a faintly dark coloration. There were very few exceptions, however, in which no dark band was noticeable.

The behavior of such a denervated area in the course of time was not without interest. In dim diffused light the denervated area was in striking contrast with the area intact, the dispersion continuing usually 2 to 3 days after the operation, and then the degree of dispersion decreased until after a week or so it reached a condition quite indistinguishable from that of a normal area. According to our records the most rapid recovery of a denervated area to a condition similar to that of an intact area took about 30 hours after operation; some areas persisted at the longest 9 days after operation in a slightly dispersed condition.

The larvae whose fins had been cut were experimented upon by transferring them from normal environment to both white-walled and black-walled vessels, and to a completely dark situation, and vice versa, the days or intervals after operation being recorded.

Generally speaking, although the results obtained were by no means clear-cut, they were as follows. If larvae with dark bands are placed in an illuminated black-walled vessel, the tint of the body as a whole becomes dark, for the change does not take place only in the denervated area if such a treatment in dark background is made within a very few days after denervation. In most cases, however, in which such a treatment is made about 7 days after denervation, that is, at about the time when the initial dark band almost fades out, the denervated band remains rather pale while the rest of the body is dark. Such a pale band standing out in the fin may assume a dark tint like that of the rest of the body, if its exposure to a dark background is greatly extended. If larvae with a dark band are put in an illuminated white-walled vessel, the band tends to become like the rest of the body, though it takes much more time for it to turn into a pale condition. But as time passes, the time required for the tint of the band to change decreases considerably, and finally, as would be expected, the difference between the color of the band and that of the rest of the body is not discernible when they are exposed to this background. A similar condition is found when larvae with denervated bands are put in complete darkness. Usually, at least a few days after the fin-cutting, the denervated band remains dark in complete darkness, the remainder of the body being pale. Some time later, however, the

band becomes as pale as the general body surface. The experiments on the larvae of *Oncorhynchus* described above appear to yield results similar to those of PARKER ('34 b) which he obtained from his experiments on adult *Fundulus*.

It must be noted, however, that there were a certain number of cases in which the above statement would not hold true. Particularly in cases when similar experiments were made on larvae younger than 20 days, the results were much more irregular. The reason why such irregular results often ensued might be explained partly by the fact that the extent of destruction produced by the initial cutting was not uniform and partly by the fact that the physiological states at different times after the cutting varied greatly in individuals. Therefore it is reasonable to think that there are factors acting independently other than those of light, darkness and backgrounds. Hence the conclusion as to the effects of such environments upon a denervated band must wait until thorough analysis of the factors can be made. Experiments as to how such a denervated band behaves when subjected to the use of various drugs will be described in the following pages.

#### ACTION OF OXYGEN AND CARBON DIOXIDE ON MELANOPHORES

It is known, though vaguely, that the oxygen content and CO<sub>2</sub> dissolved in the medium in which fishes live have some effect upon the chromatophore changes (SPEATH, '13; LOWE, '17). In this study our hope was to investigate to what extent the melanophores are related to, or controlled by the contents of oxygen and CO<sub>2</sub> dissolved, and furthermore whether they act upon the melanophore nerves or upon the melanophore cells themselves. Two groups of newly hatched and seven-day larvae respectively were placed in a vessel with a volume of 8.5 cc, which was filled with tap water and tightly covered. The melanophores of all the larvae contracted fully attaining a state of maximum concentration within 25 to 40 minutes. This process of concentration, however, was a gradual one. It was found to be true also with older larvae whose yolk had been completely absorbed. When after this procedure a complete pallor ensued, some of the larvae continued breathing, but the others did not. All of them, however, showed irregular heart beating. When they were transferred to tap water in a place devoid of illumination, they gradually returned to a moderately dark tint.

As the above preliminary test shows, it was desirable to find out

whether the oxygen depression acts directly upon the melanophore cells in producing the pallor or only upon the nerve structures concerned as such, and conversely, whether the  $\text{CO}_2$  accumulation is responsible for acting directly upon the melanophore cells or merely upon the nerve structures.

For determining the effect of  $\text{CO}_2$  on the melanophores, carbon dioxide which had been generated into boiled tap water was used.  $\text{CO}_2$  was produced by the reaction of hydrochloric acid on marbles and the  $\text{CO}_2$  gas obtained was washed by passing it through bicarbonate soda. The pH value of the  $\text{CO}_2$  saturated water prepared by this procedure is 4.2. For this experiment were used the vessels holding 70 cc. The pH value of the tap water used is 6.9. Various solutions were prepared by adding tap water to the  $\text{CO}_2$  saturated water as follows :

$\text{CO}_2$ saturated water	Tap water	pH
50 cc	50 cc	4.6
33.3 cc	66.7 cc	4.8
25 cc	75 cc	4.9

The  $\text{CO}_2$  saturated solution was found to anesthetize the gill movement immediately after immersion, and the heart beat stopped in one to three minutes. In most instances there were no reactions of the melanophores at the time the heart beat stopped, but in a very few there could be seen a tendency to contract although this was possibly not due to the action of carbon dioxide.

The second solution (50 cc of  $\text{CO}_2$  saturated solution to 50 cc of tap water): In 5 to 7 minutes this solution caused the gill movement to stop and made the heart beat very slow and irregular. The melanophores, however, tended to contract to a very slight degree. Twenty minutes later it stopped the heart beat and caused a complete concentration of the melanophores in the caudal fin. However, the melanophores in the trunk and head contracted only to a very slight degree.

The third solution (33.3 cc of  $\text{CO}_2$  saturated solution to 66.7 cc of tap water): In about 8 minutes the gill movement became weakened but the heart beat remained apparently unaffected. The concentration of the melanophores in the tail region was marked although it was unnoticeable in the regions of the trunk and head. Twelve minutes later in most instances the gill movement was paralyzed. The heart beat did not stop as yet, although the rate of the beat fell off considerably accompanied by a very irregular rhythm. The tail region became completely pale. The melanophores in the regions of the trunk and head were contracted

a great deal in some cases, only moderately in others. Twenty minutes later no marked advancement in the concentration of the melanophores was found. In some specimens the heart beat was completely arrested. After an hour or more no remarkable change was noted.

The fourth solution (25 cc of  $\text{CO}_2$  saturated solution to 75 cc of tap water): The result was almost the same as in the third solution. After seventy minutes the gill movement was completely paralyzed, but the heart beat did not stop.

These results seem to indicate that the oxygen deficiency is a primary factor in producing the concentration of the melanophores and that the  $\text{CO}_2$  accumulation in the medium does not presumably affect the melanophore reactions. To make this point certain another set of experiments was conducted in the following manner. An oxygenated solution freed from  $\text{CO}_2$  was prepared by passing it through oxygen gas into boiled tap water for 15 hours or more. Three sets of vessels each holding 70 cc were prepared as follows: (I) one with a tight cover was filled with oxygenated water; (II) the second also with a tight cover with tap water alone; and (III) the third with tap water but without any cover. Five fish 21 days old were placed in each vessel under a dim light, bright illumination being avoided in order to keep the condition originally assumed, so that the fish might be able to persist in a moderately dark coloration. One of the typical examples out of several experiments is shown in Table 2.

Similar experiments to the above were carried out with the use of either older or younger larvae than those used in Table 2. The results thus obtained were in the main the same as those above, indicating that the melanophores of the fish which were immersed in an oxygenated solution remained unchanged from 4 to 5 times as long as those in the tap water.

Test was made also with the same procedure to see how a denervated area would respond to such an environment. Oxygen deficiency caused the dark band to become pale, but usually it took much more time for the denervated area to become pale than the parts intact. Whether this was due to the blood circulation being modified by operation or due to other factors involved has not yet been determined. But it may be said that as a rule the reactivity to oxygen deficiency in bringing about the concentration of the melanophores is much greater and more rapid in the younger than in the older larvae.

SPEATH ('13) called attention to the fact that the absence of oxygen caused a contraction of the melanophores of the isolated scales not as a

TABLE 2.

*Showing a relation between the melanophore change and oxygen deficiency.*

Time in hours	I			II			III
	pH	Melanophore condition	Remarks	pH	Melanophore condition	Remarks	Melanophore condition
4 <sup>1</sup> / <sub>2</sub> -5	6.9	No change. Moderately dark including the fin.	Heart-beat and gill movement normal.	6.9	Tail completely pale. Body and head moderately pale.	Gill movement rapid. Heart-beat normal.	No change. Moderately dark.
7-7 <sup>1</sup> / <sub>2</sub>		No change. Moderately dark.			Whole body very pale.		"
9-9 <sup>1</sup> / <sub>2</sub>	6.4	No change.		5.8-5.6	Complete paling.	Gill movement almost stopped. Heart-beat weakened.	"
11	6.0-5.8	Slightly contracting.	Heart-beat and gill movement still normal.				"
13	5.6-5.4	Tail completely pale. Head and trunk very pale.	Gill movement rapid. Irregular heart-beat.				"
14 <sup>5</sup> / <sub>6</sub>	5.4	Complete paling of the whole body.	Gill movement almost stopped. Still heart faintly beating.				"

The above experiment was started at 10:10 a.m. and completed at 2:50 a.m. on the following day. I....oxygenated water with a tight cover; II....tap water alone with a tight cover; III....tap water without any cover. Further explanation in text.

specific expanding stimulus but as a passive influence. LOWE ('17) found that "oxygen is necessary for the maintenance of the expansion of the melanophores" and that "carbon dioxide produces a contraction of the pigment cells" in living trout embryos. From LOWE's statement it is not clear how much of the contraction of the melanophores is due to the action of carbon dioxide itself. According to our own results, carbon dioxide itself does not act upon the melanophores in producing the contraction, if enough oxygen be present in the solution in which the animal is immersed. Without doubt therefore it is only the depression of oxygen



that causes such a contraction of the melanophores. It may be concluded furthermore that even in the living fish the action of oxygen on the melanophores is passive, as SPEATH demonstrated in his experiments on isolated scales.

#### ACTION OF VARIOUS DRUGS ON MELANOPHOIRES

1. *Chloretone*. The larvae of various ages from the time of hatching to 40 days were immersed in different concentrations of chloretone (0.2% ; 0.1% ; 0.05% ; 0.04% ; 0.02%). The reactions of the melanophores were always expansive no matter what the ages of the larvae tested. With the stronger concentration of 0.1%, expansive reaction resulted uniformly throughout the whole body. On the contrary, with the weaker concentrations the reaction was somewhat irregular, particularly in younger larvae. Although the melanophores in the head and trunk region became expanded without exception, these in the caudal fin did not undergo such a change in certain cases observed. Irregular reactions to chloretone in cases where weaker concentrations were used seem to show that chloretone would not have any specific effect upon the melanophore cells.

40-day larvae with denervated areas in the caudal fin, which had been produced 5 days previously, were placed in a 0.05% solution. The melanophores of the denervated areas became concentrated, while those of the intact parts of the fin and the trunk region became expanded, or otherwise remained unaffected. This test was also made with 35-day larvae, whose denervated areas had been prepared 2 to 3 days previously, with similar results.

From what has been mentioned above, it seems evident that chloretone acts through the nervous system as a paralytic agent so as to cause an expansion of the melanophores. Therefore, this drug may have no direct effect upon the melanophores. WYMAN ('24 a) found that in *Fundulus* chloretone causes an expansion of the melanophores of the larval form, but does not have any effect on the embryonic melanophores.

2. *Ether*. 1.0%, 1.5%, 2.0%, and 2.5% solutions of ether were used. The immersion of newly hatched larvae in the above solutions resulted in the concentration of their melanophores in a few minutes. 2-, 5-, 20-, 27-, and 30-day larvae were also immersed in ether solutions with similar results. Throughout the experiments performed with ether solutions, however, the melanophore reaction was most prominent and rapid in the head and caudal fin, but in the trunk region such reaction was a little

less noticeable than in the parts mentioned above and frequently almost no reaction was observed. It was found that ether causes an expansion of the melanophores in the denervated area of the caudal fin as well. It may be concluded therefore that the melanophore response to ether is to expand, this being due to the paralysis of the related structures. Similarly, WYMAN ('24 a) found an expanding reaction to ether in the larval melanophores of *Fundulus*.

3. *Cocain*. The larvae at the time of hatching were placed in 0.5% and 0.05% solutions of cocain which tended to cause a concentration of the melanophores. In the younger larvae of various ages examined, the melanophores in the trunk and caudal fin were first affected after several minutes of immersion, and then the head. In some cases the melanophores in the head seemed to be hardly affected at all. The older larvae (30-, 35-day old) treated in the above manner showed that cocain had a much smaller effect upon them than upon the younger larvae and that in a 0.5% solution the melanophores in the trunk region alone contracted rapidly, while those in the head and caudal fin, remained unchanged even when the gill movement had been completely paralyzed. In general, throughout all the stages of the larvae, however, a much quicker reaction resulted in the contraction of the melanophores when they were immersed in a 0.5% than in a 0.05% solution.

VON FRITSCH ('11) found that cocain had a concentrating effect upon the melanophores in the minnow and *Carassius*, and that this concentration came through the medium of the central nervous system but not through that of the sympathetic system. WYMAN ('24 a), working upon the *Fundulus* embryos, states that cocain causes a primary concentration of the melanophore cells, which is soon followed by an expansion of the cells. In this he is in agreement with the findings of LOWE ('17) in regard to trout embryos. LOWE suggests that such action is due to affection of the nerve endings of the pigment cell.

The writer has failed to confirm this findings of LOWE and WYMAN in that he has found that cocain acts first in a concentration of the cells, which is then followed by expansion due to paralysis of the cells setting in. His own results, however, indicate that cocain has no definite effect on the melanophores in parts other than the trunk region and that no paralytic expansion follows a lapse of time.

4. *Strychnine*. The larvae of various ages, newly hatched, 7-, 12-, 34-, and 42-day larvae, were immersed in 0.05% and 0.005% strychnine solutions. In several minutes the 0.005% solution of strychnine caused

a very great or complete concentration of the melanophores, notwithstanding the gill movement remained unaffected for 5 hours or more. In a 0.05% solution paralysis of the gill movement occurred in about 10 minutes after immersion. In a few minutes this solution caused a complete concentration of the melanophores. 30 to 60 minutes later the larvae were replaced in tap water with their melanophores concentrated. 5 hours later, even when the larvae were immersed in a 0.005% solution, no apparent paralytic expansion of the melanophores followed by a primary concentration, such as LOWE ('17) and WYMAN ('24 a) described, was produced.

It was found that strychnine acts upon the younger larvae much more vigorously than upon the older larvae, and that it has only a slightly smaller effect on the melanophores in the head region than on those in the other portions of the body.

5. *Nicotin*. When the oldest larvae (45-day) were immersed in a 0.04% solution of nicotin, the gill movement stopped in from one to two minutes. It however was found to have no effect on the melanophores. Immediately after the younger larvae such as those just hatched or such as were 10-days old were immersed in the same strength of solution the melanophores contracted. Such a contraction of the cells occurred before the gill movement stopped but the heart continued to beat. Following the contraction of the cells, possibly in 7 to 10 minutes, they began to expand gradually, with the exception of those in the trunk region. Even after two hours the melanophores in the head and caudal fin remained expanded, although in the trunk region some were only slightly expanded, the others somewhat contracted. When the larvae treated above were replaced in tap water, they were all still alive.

The melanophores of the young larvae when immersed in a 0.01% solution of nicotin began to contract and in a few minutes assumed a state of complete contraction. The melanophores in the caudal fin appeared to be the ones most rapidly and prominently affected. After one hour or so the contracted melanophores tended to expand again. But even 2 hours later, they had not expanded fully. Such a process of expansion continued for 5 hours without being completed. This same solution had no effect on the melanophores of the older larvae, except that in the caudal fin only a slight contraction of the cells was produced. After 15 minutes those contracted melanophores in the caudal fin began to expand again, but still no change was elicited in the head and trunk regions. These remained unchanged for several hours.

Again the larvae of various ages were immersed in a 0.005% solution of nicotin. The melanophores of the younger larvae began to contract immediately, and remained completely contracted for 3 hours or more. After this they gradually expanded, and most of them died in a state of expansion about 10 hours after immersion. In the older larvae the solution had no effect on the melanophores, although in a very few cases there occurred a very slight contraction of the melanophores, which was followed by a slight expansion, resulting in a return to the original coloration.

The results recorded above show that nicotin causes a primary contraction of the cells and then a secondary paralytic expansion, the time necessary to bring about such a paralysis depending upon the strength of the concentration of the solution. LOWE ('17) stated that in the trout embryos the action of nicotin was on the sympathetic system of the pigment cells, for a primary contraction of the cells was followed by a paralytic expansion. The writer's own results seem to be in agreement with that of LOWE.

6. *Adrenalin.* Embryos in which the melanin granules began to appear were taken out of the shell and immersed in adrenalin solution (Sankyo Company's preparations), 1:10,000 and 1:100,000. Such embryos, the melanophores of which had just begun to appear on the head, reacted by complete concentration within several minutes. Larvae at the time of hatching were treated with adrenalin solutions of various concentrations, 1:10,000, 1:100,000 and 1:150,000. Shortly after they had been placed in 1:10,000 and 1:100,000 solutions, the melanophores began to contract uniformly all over the body. In the 1:150,000 solution contraction of the cells situated in the tail region and the caudal fin took place in several minutes, but in the head and trunk regions they appeared to be affected much more slowly, no complete contraction of the melanophores being evident.

Younger (10-day) and older (35-, 40-day) larvae were also immersed in adrenalin solutions prepared as above, with almost the same results as those obtained with the newly hatched larvae. Especially, when the older larvae were treated with the 1:150,000 or weaker concentrations of adrenalin, the melanophores in the head and trunk regions were affected gradually but to irregular extent, while, on the contrary, in the caudal fin there occurred a complete contraction of the melanophores. Although adrenalin acts upon the melanophores as a contractile force no matter what the age of the larvae may be, it should be said that the younger the larvae and the stronger the solutions used, a much quicker and clear-

cut reaction ensues.

Dark-tinted larvae were injected with 0.1 to 0.2 cc of adrenalin chloride, one part to 10,000 of water. In a very few minutes they became completely pale. The pale condition continued for more than four hours after injection. Using one part to 50,000 of water, complete paling also occurred but the persistence of such a condition appeared to be somewhat of shorter duration than when the stronger solution was used, say, nearly three hours. In a similar manner a number of dark-tinted larvae were injected in concentrations of one part to 100,000 and one part to 500,000. The melanophores in the head and caudal fin came to be fully concentrated in several minutes, but in the trunk region some did not contract so well. After one to two hours all of them assumed a moderately dark coloration again. In much more diluted concentrations of adrenalin solution the results were not decisive at all, though some slight tendency to cause a pallor could be seen even with injections of one part to 1,000,000 of water.

Older larvae with dark bands in their caudal fins, produced by the cutting of their rays, were injected with the various solutions of 0.1 to 0.2 cc of adrenalin mentioned above. Such dark bands responded in a similar manner to the intact portions of the body. So we might say that, all of the melanophores became uniformly concentrated. (See p. 41).

7. *Acetylcholine*. Fifteen- and forty-day larvae were immersed in 0.05%, 0.005% and 0.0005% solutions of acetylcholine chloride (Schering-Kahlbaum Company's preparations) for two and a half hours, with no results. In younger larvae, however, the melanophores in the trunk region alone appeared to expand very slightly irrespective of the backgrounds to which they were subjected. The larvae at the time of hatching were immersed in acetylcholine of the varying strengths mentioned above, and in no case did the solutions cause any appreciable change in the melanophores.

The body cavity of thirty- and forty-day larvae were injected with a 0.2 cc of 0.06% solution. In several minutes the melanophores tended to expand. However, after this the results became irregular and in most cases the larvae treated died. The same experiment was repeated several times with no confirmative results. Injection of a 0.006% solution of acetylcholine, however, caused the darkening of all the specimens treated in 10 to 20 minutes. The darkening started first in the head, and finally included the trunk and the caudal fin. In most cases this dark state lasted nearly seven hours after injection. When a 0.0006% solution was

injected, the results were somewhat irregular, although there seemed to be a tendency to darken slightly. Such a slight darkening, if present, appeared to fade out rather quickly. (See pp. 41-42).

8. *Pituitrin*. Contracted melanophores of 15- and 40-day larvae were exposed to 1%, 0.1% and 0.01% pituitrin solutions (Parke, Davis Company's preparations). A very slow expansion of the melanophores occurred. After one hour and a half all the melanophores expanded to a greater degree, but those in the caudal fin revealed but a very slight expansion. After eight hours or so the expanded melanophores began to contract again. In the newly hatched larvae this expansion of the melanophores was much more rapid. The melanophores in the caudal fin, however, appeared rather to contract. Experiments on a number of larvae of varying ages seem to show that pituitrin acts upon the larval melanophores by way of inhibiting the loss of color and later causing the paling of the once expanded melanophores.

When a 0.2 cc of 0.01% solution of pituitrin was injected hypodermically, the contracted melanophores of the older larvae began to expand in several minutes. The head and the trunk regions were the first to darken and a little later the caudal fin was similarly affected, though not very prominently. Darkening of the head and trunk regions reached a maximum in 10 to 15 minutes. It was proved that in the case of injection the melanophores in the caudal fin were affected just as slightly as when they were placed in the solution itself. In most cases the darkening lasted more than five hours. A certain number of the larvae died in the darkened state. Injection of a 0.001% solution was performed with similar results, without, however, having very noticeable effects. (See p. 42).

9. *Thyroideum*. The larvae of different ages from the time of hatching to 40 days were immersed in 0.1% and 0.01% solutions of thyroideum (*Thyroideum siccum*; Parke, Davis Company's preparations). In no case did it cause any well-defined change of the melanophores, even after several hours of immersion.

Injection of 0.05% and 0.005% solutions of thyroideum did not have any effect on color change, any noticeable difference in their condition being due rather to the backgrounds to which the larvae were exposed.

## DISCUSSION OF THE RESULTS

Before attempting to decide whether or not the response of the melanophores from the first are under control of the nervous system, we must find out at just what stage of development the melanophores of the fish are able to respond to light, darkness and backgrounds. The experiments performed on *Oncorhynchus keta* with a view to discovering changes in the melanophores under the different environments to which the fish were exposed, from the time of the first appearance of the melanophores in the embryos showed that when put on an illuminated light background the youngest melanophore cells newly formed contracted, but when put either in complete darkness or on an illuminated dark background, practically no change of the melanophores took place. This point will be discussed later.

WENCKEBACH (1886) reported that in embryos of *Pleuronectes* bright illumination causes the pigment cells to expand and darkness makes them contract. BECHER ('29) saw that in *Cregonus fera* and two species of *Salmo* the melanophores are responsive to light in an embryonic stage earlier than the time of hatching.

According to the results of DUSPIVA ('31), to which we have already referred, the embryonic melanophores in *Perca* become active about as soon as the processes of the cells are formed, expanding in the light and contracting in the dark. The same author claims that the same is the case for the embryo of *Salmo salvelinus*. More recently TOMITA ('36) stated that the embryo of the Paradise fish upon which he worked responds with melanophore change to the stimuli of light soon after the first melanin granule formation. Work on other fishes also leads us to believe that, whatever the reactions may be, the capability of reaction in melanophores in an early embryonic stage is established as firmly as it was in the present observation upon the salmon. In this respect, however, it may be noted that there is a difference from the reactions found in amphibians in which, according to LAURENS ('15, p. 633), "the melanophores of very young larvae do not react to light, darkness and backgrounds, until certain stages of development have been reached".

Although DUSPIVA ('31) showed that the first reaction of the melanophores is to cause an expansion in light and a contraction in darkness, a view substantiated by the recent results of the work of TOMITA ('36), the case of salmon, as far as the present observation goes, is different. According to the writer's own results, bright light causes a contraction

of the melanophores from the very beginning where the pigment cells were formed just over the brain, quite in the same way as in those of the adult. A very similar result has been reported by PARKER ('36) in an ovoviparous dogfish.

As for reactions to darkness, what is shown by the salmon experimented upon is also different from the reactions reported by many writers. The present results indicate that only after attaining a certain stage of development did the larvae become considerably lighter in color in complete darkness, notwithstanding that they remained unchanged in the state of a slightly dark tint until a certain larval stage of development had been reached. Prior to the stage mentioned above, the specimens, speaking generally, were kept in a state freed from any possible stimulation that might cause a change in the melanophores, having been placed in an indifferent background devoid of illumination. Although such a condition, in which the fish become pale after a certain larval stage of development seems to be like what is called the primary phase in the other fishes studied by WENCKEBACH, DUSPIVA and TOMITA, it points also to differences in that the salmon does not respond to darkness in its early larval stage and no transition from an expanded to a contracted state is observed even after the yolk has been completely absorbed and after it has acquired the capability of reaction to light. On the contrary, that the larvae of *Oncorhynchus* assume a pale tint in complete darkness may be regarded as an identical phenomenon to those reported in regard to a certain number of adult fishes; for instance, by PARKER and LANCHNER ('22) in their investigations of *Fundulus*, by WYKES ('36) in his work on *Gobius pagenellus* and *G. ruthensparri*, *Ctenolabrus rupestris*, *Lepadogaster gouani* and *Phoxinus phoxinus*, and by WARNING ('36) in his studies of the dogfish, and so on.

It must be borne in mind that the present specimen studied apparently possesses a visual system of a far more advanced development in general at the time when the melanophore system makes its first appearance than those upon which DUSPIVA and TOMITA worked. A possible explanation of this phenomenon therefore is that in *Oncorhynchus* the visual system may be functional in some way at the time when the melanophore cells are formed, so that bright light causes a contraction of the melanophores just as in many adult forms, a reaction quite different from those in fishes which possess the melanophores in their very early stage of development. Similarly it is quite possible to assume that there is present a functional differentiation in the developmental process of the retinal elements in their



relation to light and darkness. If this be accepted, then the retinal elements in *Oncorhynchus* which are related to darkness or black, accordingly might not function until long after those elements which are related to brightness or white become functional. It follows therefore that the larvae remain unchanged in darkness until a certain stage of development has been reached, while, on the contrary, light illumination causes a contraction of the youngest melanophores. This view may receive additional substantiation from the experiment in an illuminated black background, in which the larvae did not show any apparent change of the melanophores before they had attained the stage of development mentioned above. As the present experiments indicate, thus, there exists at least in *Oncorhynchus* no distinction between what is called the primary and the secondary phase during the course of development, its responses being like those of the adult, except that in a very early stage of development darkness does not induce the melanophore change. This difference seems to be due only to the fact that there is a variation in developmental process between the visual system and the melanophore cells.

Although any final conclusion must be reserved for the present, we should now consider the two photoreceptor systems controlling the melanophore responses, of which one is dermal and the other visual. According to the suggestion of SAND ('35), a purely dermal response to light is to be found common to all the teleosts. WYKES ('36) suggests however that "the dermal response is variable in its incidence not only in different groups but also at different stages in the life history". (p. 85). In the case of the larvae of *Oncorhynchus*, we should not exclude the presence of the dermal response in discussing the mechanism of the melanophore change. It is proved that enucleation of the eyes has an effect on the melanophores causing an expansion uniformly throughout the larval stage, even at the time of hatching. However, neither illumination nor darkness gives rise to any change of the expanded melanophores in the blinded larvae until a certain stage is reached. These observations are opposed to the view held by DUSPIVA and TOMITA that when the enucleation is made in the primary phase the melanophores of the young eyeless larvae are responsive to light and darkness in the same way as when the eyes are intact, but, if made in the secondary phase, the melanophore reactions revert to the primary phase in that they contract in darkness and expand in bright illumination. In the case of *Oncorhynchus*, however, it is only after its later larval stage that the melanophores of the blinded fish tend to contract slightly both when illuminated and when in complete darkness.

This is supported by the similar results obtained from the experiments with the denervated band of the fin (p. 27). The facts above described and those of unmentioned and fragmental observations suggest that in *Oncorhynchus* the purely dermal response to light may be regarded as of little importance in producing the melanophore reactions in the young larval stages, but after a certain larval stage it begins to function to some extent. This question however is one which deserves further investigation, together with that of the exact location of dermal photoreceptors and their development.

However much we may desire to do so, space does not permit us here to discuss the results obtained from the experiments concerning the action of drugs upon the melanophores. Mention however should be made of the reactions which resulted when the larvae were subjected to the action of adrenalin, acetylcholine and pituitrin.

There is no room for doubt that adrenalin has a direct effect upon the melanophores as has been demonstrated by numerous investigators. WYMAN ('24 a) found that in *Fundulus* the melanophores of five-day embryos were completely contracted by the use of adrenalin. DUSPIVA ('31), working on the embryos of *Salmo*, stated that as soon as the melanophore cells appear, they are controlled by a double innervation, in view of the fact that in the youngest embryo he experimented on with the use of adrenalin a contraction of the melanophores took place. According to our results, the melanophores when they first appear in the embryos, those of eyeless larvae, and those of a denervated area as well as those of the remainder of the body contract by the use of adrenalin. Therefore, it is evident that adrenalin acts upon the melanophores as a sympathetico-mimetic drug in calling forth their contraction. The exact site of this action, whether on the nerve endings which innervate the cells or on the cells themselves, however, requires further study.

Results concerning the effect of acetylcholine on fish melanophores are not all in agreement. GIERSBERG ('30) found that in *Phoxinus* acetylcholine induces a deep darkening. PARKER ('31) reports that injection of acetylcholine alone causes the darkening of the fish, but in most cases death occurs when a concentration strong enough for producing this color change is used. But when physostigmine is employed as a protective agent, acetylcholine induces the lightening of the skin (PARKER, '34 a). This would make it doubtful, to our minds, whether physostigmine did not have some other effect upon the melanophores different from that when acetylcholine alone was injected. SMITH and SMITH ('34) state that the

chromatophores of *Scorpaena* are not affected by acetylcholine either in case of immersion or injection.

According to the results of the investigations of DUSPIVA ('31), when he worked upon the embryos of *Salmo*, the injection of acetylcholine had the effect on the larval melanophores of calling forth a darkening of the body, while adrenalin caused paling. He came to the conclusion that even in the youngest melanophores of the fish on which he worked there was a double innervation, sympathetic and parasympathetic, and hence that it was possible to demonstrate histologically the nerve endings at the same time. Like those of GIERBERG ('30), SMITH ('31) and DUSPIVA, our own results favor the view that fish melanophores are governed by a double innervation, which is opposed to the conception held by some other writers that they possess only a single innervation. However, the possibility that some part of the process is under the control of humoral agencies "which have free access to the integumentary melanophores" should never be excluded.

In the present experiment it was obvious that injection of pituitrin tended to cause an expansion of the melanophores in all the larvae studied. This was quite in agreement with the results found by PARKER ('34) in his study of catfish. Without discussing the matter further, our experiments indicated that in the case of larvae the pituitary gland is one which influences the melanophore change in calling forth an expansion by acting as "a dispersing neurohumor" if we may use an expression formulated by PARKER ('32).

#### SUMMARY AND CONCLUSIONS

1. The first appearance of the melanophore cells on the body surface in *Oncorhynchus keta* is in the very late embryonic stage, nearly ten days prior to hatching, which takes about two months after fertilization in normal conditions, temperature ranging from 4° to 8°C.

2. At about the time when the melanophores make their first appearance in the embryos, they are responsive to illumination which causes the contraction of the pigment cells. This reaction is the same throughout all the larval stages.

3. Complete darkness has no effect on the change of the melanophores until a certain larval stage of development is reached, that is, about 20 days after hatching. When this period has passed, it causes paling very much like that observed in adults. Similarly, larvae on a black background,

whether illuminated or not, do not show any apparent reaction until a certain period of the larval stage. After this period, they are decidedly dark in tint. Therefore, it is suggested that in the early stages of larvae they are not sensitive to black.

4. Enucleation of both eyes causes an expansion of the melanophores in all the larval stages.

5. A denervated area produced in the caudal fin by the cutting of the nerves shows a faintly dark coloration which is caused by a very great expansion of the melanophores. The degree of expansion and its persistence vary with individuals. In most cases the dark band fades out within a week. The effects on the denervated area of varying backgrounds are described above, but no generalization has been worked out.

6. We may conclude that the distinction between the primary and secondary phases in the change of the melanophores in the larval stages which is found in amphibians and in some other fishes, cannot be regarded as present in *Oncorhynchus*, the primary phase in its ontogeny being omitted. We would suggest that such a distinction may be determined by differences in development in the visual and melanophore systems. This distinction varies according to the species studied.

7. It is suggested that in *Oncorhynchus* the dermal response to light is of much less importance than the retinal, at least in the young stages, but possibly later it develops as well.

8. Oxygen deficiency causes a contraction of the melanophores, but carbon dioxide has no apparent effect on them.

9. Chloretone and ether cause an expansion of the larval melanophores. Cocain, strychnine and nicotin all cause a contraction, but in the case of nicotin the primary contraction is apparently followed by a secondary paralytic expansion.

10. Adrenalin, in either case of immersion or injection, causes a contraction of the embryonic and larval melanophores. Acetylcholine, when injected alone, has an effect on the larval melanophores in calling forth the darkening of the body. Pituitrin, also, tends to cause an expansion of the larval melanophores. Thyroideum has no effect upon them.

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# ANNELIDA POLYCHAETA IN ONAGAWA BAY AND ITS VICINITY

## I. POLYCHAETA SEDENTARIA

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(With Plate II and twelve text-figures)

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The following account of the Polychaeta Sedentaria is based upon a collection made by the authorities of the Onagawa Oceano-chemical Institute of the Tôhoku Imperial University from Onagawa Bay and its vicinity in the summer of 1935.

Here follows a list of species.

Family Ariciidae

*Aricia fimbriata* MOORE

Family Spionidae

*Prionospio pinnata* EHLERS

Family Magelonidae

*Magelona japonica* OKUDA

Family Chaetopteridae

*Spiochaetopterus* sp.

Family Cirratulidae

*Audouinia comosa* (MARENZELLER)

Family Chloraemidae

*Stylarioides plumosa* (O. F. MÜLLER)

*Stylarioides eruca* (CLAPARÈDE)

*Brada villosa* (RATHKE)

Family Arenicolidae

*Arenicola cristata* STIMPSON

Family Maldanidae

*Praxillella affinis* (SARS)

Family Amphictenidae

*Pectinaria (Cistenides) hyperborea* MALMGREN

Family Terebellidae

*Amphitrite ramosissima* MARENZELLER

*Amphitrite cirrata* (O. F. MÜLLER)

*Amphitrite rubra* (RISSO)

*Polymnia nebulosa* (MONTAGU)

*Pista elongata* MOORE

*Thelepus setosus* (QUATREFAGES)

#### Family Sabellidae

*Potamilla reniformis* (O. F. MÜLLER)

*Potamilla myriops* MARENZELLER

*Sabella* sp.

#### Family Serpulidae

*Hydroides uncinata* (PHILIPPI)

*Pomatoleios crosslandi* PIRELL

*Salmacina dysteri* (HUXLEY)

*Spirorbis nipponicus* OKUDA

Of 24 species herein recorded only 8 are newly added to the marine fauna of Japan. A general geographical review of the polychaete fauna in these regions must be postponed until the completion of the investigation of the second half of the collection, i. e., Polychaeta Errantia, but, so far as observed on the present materials, it can be concluded that the polychaete fauna of the Onagawa Region is composed partly of boreo-arctic forms, such as *Pectinaria hyperborea* or *Brada villosa*, and partly of tropical or subtropical forms, such as *Pomatoleios crosslandi* or *Amphitrite rubra*.

The brief description of each species is intended to afford a rough determination of the local fauna. The dichotomous key of families and generic characters herein adopted have been mainly founded on those given by FAUVEL, GRAVELY and MONRO.

Before proceeding further my hearty thanks are due to the gentlemen of the Onagawa Oceano-chemical Institute who placed the valuable collection at my disposal and also to Prof. TOHRU UCHIDA for his kind guidance in the course of the investigation.

#### Key to Families found in the Onagawa Region

1. Body divided into distinct regions. ....6
- 1'. Body not divided into distinct regions. ....2
2. Palpi long, tentacle-like. ....3

- 2'. No tentacle-like palpi. Prostomium with a keel or bordered cephalic plate. An anal plate or an anal funnel with cirri. No branchiae. Ventral tori with sigmoid hooks. . . . . Maldanidae
3. Two long tentacular palpi on prostomium. . . . . 4
- 3'. One or more pairs of palpi inserted on the anterior segments. Branchiae simple, filiform, inserted above the notopods. Prostomium, conical, without processes. Capillary and acicular setae. . . . . Cirratulidae
4. Two palpi and two groups of branchiae retractile into a buccal funnel. Bristles of first chaetigers prolonged to form a cephalic cage. Body thickly papillated. . . . . Chloraemidae
- 4'. Two long grooved palpi not retractile into the mouth. No cephalic cage. . . . . 5
5. Palpi without suckers. Pedal lamellae erect. Dorsal branchiae cirriform. Hooded hooks present. . . . . Spionidae
- 5'. Palpi with sucker-like papillae. Branchiae absent. Prostomium flattened, spoon-shaped. . . . . Magelonidae
6. A terminal branchial tuft with numerous filaments bearing secondary processes. Prostomium indistinct. Uncini-dorsal in the thoracic region, ventral in the abdominal region. Tube membranaceous or calcareous. . . . . 11
- 6'. Without terminal branchial tuft. . . . . 7
7. Large flattened chaetae (paleae) forming an operculum closing the tube. Two pairs of anterior branchiae. Caudal region small, foliaceous, with hooks at the base. Tube of sand, conical, free. . . . . Amphictenidae
- 7'. Without opercular bristles. . . . . 8
8. Prostomium conical or blunt without processes. Branchiae on many segments. . . . . 10
- 8'. Prostomium more or less distinct. One pair of tentacle-like palpi or numerous tentacular filaments. . . . . 9
9. Prostomium with or without two small tentacles. Two long grooved palpi. Two to three markedly dissimilar regions, the anterior short with uniramous feet bearing special chaetae in 4th chaetiger. Posterior notopods erect. Uncini pectinate. . . . . Chaetopteridae
- 9'. Without tentacles. A cephalic veil and numerous tentacular filaments not retractile. Ventral tori with pectinate uncini. Prostomium indistinct. Branchiae arborescent or rarely sub-



- ulate, inserted on the first segments, sometimes absent. .... Terebellidae
10. Without uncinigerous tori. Serrated capillary setae and acicular hooks. Parapodia and branchiae conspicuous and erected on the back in the abdominal region. .... Ariciidae
- 10'. With uncinigerous tori. Anterior region abranchiate; middle with dorsal, arborescent, non-retractile branchiae; often achaetous and abranchiate caudal region. .... Arenicolidae
11. Without operculum and without thoracic membrane. Tube membranaceous or mucous. .... Sabellidae
- 11'. Usually with an operculum. Thoracic membrane present. Tube calcareous. .... Serpulidae

### Family Ariciidae

#### Genus *Aricia* SAVIGNY

Prostomium conical. Buccal segment achaetous. A pair of erect lanceolate branchiae on all except a few anterior segments. Thoracic region depressed, flattened, and abdomen rounded, cylindrical. Thoracic dorsal ramus with an erect, rarely fimbriate, dorsal cirrus and a bundle of crenate capillary setae; in the ventral ramus there are pad-like post-setal lamellae with vertical rows of stout bristles and podial papillae. Transverse vertical rows of subpodial papillae may be present on a few segments or may be absent entirely. In abdomen, an erect dorsal cirrus, capillary setae and forked setae, sometimes an intermediate cirrus. Ventral ramus bilobed, with capillary setae and a ventral cirrus. Dorsal sense organs anchor-shaped.

#### *Aricia fimbriata* MOORE

(Pl. II, Fig. A)

*Aricia fimbriata*: MOORE, 1903. p. 464, pl. 24, figs. 31-35; OKUDA, 1937, b, p. 99, figs. 1-2.

*Occurrence*: Okachi Bay and Ayukawa Bay. Collected by dredging. Sp. No. H. 94 and H. 104.

*Specific characters*: Body large, depressed in the thoracic region, semi-cylindrical in the abdominal region. Prostomium conical. Thoracic setigerous segments 16. The 17th and 18th chaetigers, though transitional in form, resembling those of the posterior region more closely. Branchiae begin on 5th setigerous segment. In the mid-thoracic region dorsal rami

bearing dorsal cirri with 7-8 fimbriated marginal papillae, and ventral rami with foot-papillae, vertical row of subuluncini, genuine hooks and capillary setae. From the 13th to 16th setigerous segments 4-5 spear-headed spines occurring in each neuropod. Subpodal papillae present from the 16th to 21st or on some of these. Intermediate cirrus absent. In the abdominal region capillary setae and forked setae in dorsal rami.

*Remarks:* The species is distinctly characterised by the possession of the thoracic fimbriated dorsal cirri.

*Former localities in Japan:* Suruga Bay and North Japan (MOORE).

### Family Spionidae

#### Genus *Prionospio* MALMGREN

Prostomium short, rounded. No frontal peaks and occipital tentacles. Eyes present. Two long palpi. Branchiae 3 to 11 pairs, often pinnate. Dorsal and ventral setae capillary. Dorsal and ventral hooded crochets.

#### *Prionospio pinnata* EHLERS

*Prionospio pinnata:* FAUVEL, 1932, p. 173; OKUDA, 1937 a, p. 247, fig. 22.

*Occurrence:* Tako-Shima. Collected by dredging. Sp. No. H. 95.

*Specific characters:* Prostomium rounded in front. There are 2 membranaceous prostomial wings. Branchiae 3-4 pairs, pinnate, beginning on the first setigerous segment. Sometimes a transverse crest occurring between the first one or two setigerous segments.

*Remarks:* A single ill-preserved specimen devoid of the posterior region measures 26 mm. Two eyes. A well marked transverse ridge on the first setigerous segment. Ventral crochets appearing from the 9th chaetiger. The species is recently recorded by the author (1937 a) from the same locality above described.

### Family Magelonidae

#### Genus *Magelona* O. F. MÜLLER

Body divided into two regions. Prostomium oval or horn-shaped. Two long palpi with sucker-like papillae. No branchiae. No eyes. Two anal cirri. Parapodia biramous. Dorsal capillary setae simple. Hooded crochets on both dorsal and ventral rami in abdominal region.

**Magelona japonica OKUDA**

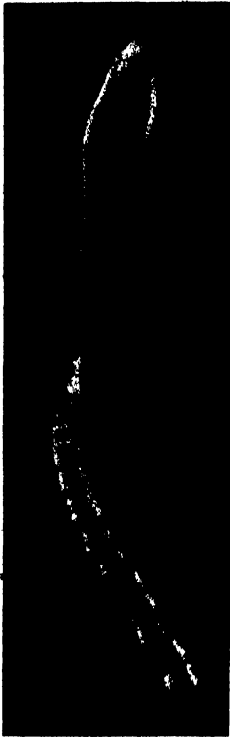
(Text-fig. 1)

*Magelona japonica*: OKUDA, 1937 a, p. 247, figs. 23-24.*Occurrence*: Tako-Shima. Collected by dredging. Sp. No. H. 124.

*Specific characters*: Prostomium horn-shaped. A pair of long, slender palpi with fine crowded papillae. In anterior region dorsal and ventral postsetal lobes slender, without processes above setigerous rami. Bristles in both dorsal and ventral rami fine, winged and capillary. Ninth chaetiger bearing also fine capillary setae bordered on one margin. Dorsal and ventral lobes in abdominal region foliaceous, lanceolate, both similar in shape and equal in length. Dorsal and ventral hooded crochets, with 2 transversely arranged small teeth above a main fang. Deep purple pigment occurring from the 5th to the anterior border of the 8th setigerous segments.

*Remarks*: Only a fragment was collected. The species was first recorded from the Korean Archipelago. The present specimen is closely allied to the species in shape of the prostomium, in the parapodium and in the characteristic coloration of the body.

*Former locality in Japan*: Jinsen, the Korean Archipelago.



Text-fig. 1. *Magelona japonica* OKUDA. Dorsal view of anterior body.  $\times 6$ .

**Family Chaetopteridae****Genus *Spiochaetopterus* Sars**

Body slender, divided into three regions. Two long tentacles. Anterior region with uniramous feet bearing oar-shaped setae. Peculiar spines on the 4th setigerous segment. Middle region with number of biramous feet, dorsal rami foliaceous, lobed, and ventral rami bilobed. Posterior region with biramous feet, dorsal rami cylindrical; ventral rami uncinigerous. Tube horny, translucent, cylindrical and annulated.

**Spiochaetopterus sp.**

**Occurrences:** Oôra Inlet. Collected by dredging. Sp. No. H. 110.

**Remarks:** Only a fair number of empty tubes. They are slender, horny, rigid, cylindrical with rings, recalling those of *Spiochaetopterus typicus*.

**Family Cirratulidae****Genus Audouinia QUATREFAGES**

Body long, subcylindrical, tapering at both ends, prostomium conical. Tentacular filaments appearing on one or more segments behind the first branchiae bearing segment. Branchial filaments from the first segment to nearly the last ones. Capillary setae and hooks in both rami.

**Audouinia comosa (MARENZELLER)**

(Pl. II, Fig. B)

*Cirratulus comosa*: MARENZELLER, 1879, p. 39, pl. 6, fig. 7; EHLERS, 1920, p. 62

*Audouinia comosa*: FAUVEL, 1933, p. 46; 1936, p. 73.

**Occurrence:** Oôra Inlet and Onagawa Harbour. Collected by dredging. Sp. No. H. 75 and H. 125.

**Specific characters:** Body large. Branchial filaments from the first setigerous segment. Tentacular filaments in dense clusters on the 5th-6th or 6th-7th setigerous segments. Distance between the point of branchiae-insertion and dorsal ramus shorter than the distance between the two rami. Capillary setae in every foot. Ventral hooks relatively slender, about 4-5, first appearing on about 25th neuropod. Dorsal hooks, about 3-5, from about 50th notopod.

**Remarks:** A single rather small specimen measures about 28 mm. As already mentioned by FAUVEL the species is closely allied to *Audouinia anachloastus*. The species is widely distributed in Japan as also in tropical seas. EHLERS recorded the species from Amboina.

**Former locality in Japan:** Sêto, Wakayama Prefecture (FAUVEL).

**Family Chloraemidae****Genus Stylarioides DELLE CHIAJE**

Body elongated, cylindrical or club-shaped, coated with papillae. Two stout palpi. Branchiae filiform, born on a retractile stalk. Bristles on

the first few chaetigers directed forward to form cephalic cages. Dorsal bristles long, capillary and annulated. Ventral bristles behind the first segments simple or rarely pseudo-compound, hook-like. Acicular setae slender. Blood green.

***Stylarioides plumosa* (O. F. MÜLLER)**

(Pl. II, Fig. C)

*Stylarioides plumosa*: FAUVEL, 1927, p. 116, fig. 41, a-g; HASSE, 1914, p. 187.

*Stylarioides borealis*: MOORE, 1903, p. 487.

**Occurrence**: Oûra Inlet. Collected by dredging. Sp. No. H. 126.

**Specific characters**: Body elongated, cylindrical, gradually tapering backwards. Skin papillae rather short, cylindrical or globular. Two stout palpi. Buccal siphon short. Branchiae slender, few in number (8 in all). Cephalic cage formed by setae of the first three setigerous segments. These bristles are long and ringed. Sigmoid crochets accompanied by fine acicular setae first appearing from the 4th chaetiger. Anus terminal. About 30-70 mm long for about 50-70 segments.

**Remarks**: Three rather small specimens were collected, of which the largest one measures 40 mm for 47 setigerous segments. The body is coated with sand particles. MOORE recorded the species from North Japan as *Stylarioides borealis*, which is considered to be synonymous with the present species. In Akkeshi, Hokkaido, the species commonly occurs.

**Former locality in Japan**: North Japan (MOORE).

***Stylarioides eruca* (CLAPARÈDE)**

(Text-figs. 2-3)

*Stylarioides eruca*: FAUVEL, 1927, p. 119, fig. 42, h-l; 1934, p. 49.

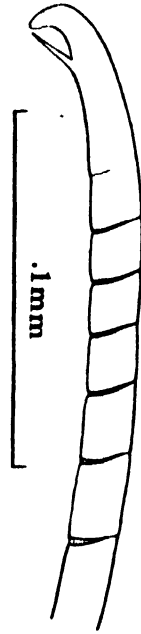
**Occurrence**: Izushima Harbour. Collected by dredging. Sp. No. H. 120.

**Specific characters**: Body thickly coated with sand grains. Long, cylindrical papillae arranged in regular longitudinal rows. Cephalic cage formed by setae of the first three setigerous segments, long, slender, not iridescent. Branchiae, filiform, variable in number, inserted on a short peduncle. Ventral setae bidentate. About 40-60 mm for 60-80 chaetigers.

**Remarks**: A single specimen measures 30 mm for 45 chaetigers. The body surface was densely coated with sand particles. This is the first record of the species from Japan.



Text-fig. 2. *Stylarioides eruca*  
(CLAPARÈDE). Entire body.  $\times 2$ .



Text-fig. 3. *Stylarioides eruca*  
(CLAPARÈDE). Ventral seta.

### Genus *Brada* STIMPSON

Body cylindrical or fusiform. The setae of the anterior segments do not form a marked cephalic cage. Two stout palpi. Branchiae cirriform, retractile, in two clusters. Dorsal setae capillary, annulated; ventral setae simple, stouter. A single pair of nephridial papillae protruding on the 4th or 5th setigerous segment.

#### *Brada villosa* (RATHKE)

(Pl. II, Fig. D)

*Brada villosa*: FAUVEL, 1927, p. 121, fig. 43, c-1; 1934, p. 49; HAASE, 1914, p. 203.

**Occurrence:** Myozin-Maye, Okachi Bay. Collected by dredging. Sp. No. H. 135.

**Specific characters:** Body fusiform. Dorsal skin papillae large, clavated; ventral papillae very small and short. Around the base of the dorsal and ventral setae tuft, the papillae are long and cylindrical arranged in rosette-form. One pair of small nephridial papillae on the ventral side of

the 5th setigerous segment. Two large palpi. Branchiae numerous. Bristles of the first setigerous segment slender, few in number; they do not form a cephalic cage. From the 2nd chaetiger backwards, dorsal bristles shorter, ringed. Ventral hooks curved, articulated. About 10-40 mm for 12-35 chaetigers.

*Remarks:* A single complete specimen measuring 20 mm for 28 chaetigers. The species which is widely distributed in the boreal regions is now recorded from Japan for the first time.

#### Family Arenicolidae

##### Genus *Arenicola* LAMARCK

Body cylindrical, often swollen anteriorly. Each segment divided into annuli. Branchiae pinnate or branched, variable in number and lacking on anterior segments. Prostomium small or moderately well developed, bounded posteriorly by the nuchal organ. No tentacles nor palpi. Neuro-podia transversely thickened, bearing a vertical row of crochets and conical notopodia with capillary setae. One or more pairs of oesophageal pouches. There are five to thirteen pairs of nephridia. A pair of peristomial otocyst, sometimes absent.

##### *Arenicola cristata* STIMPSON

*Arenicola cristata:* FAUVEL, 1927, p. 163, fig. 57, o-r.

*Occurrence:* Kiriga-Saki and in front of the Institute, sandy bottom. Sp. No. H. 72 and H. 73.

*Specific characters:* Body cylindrical, swollen anteriorly with 17 setigerous segments. Branchiae pinnate in 11 pairs. Well-marked prostomium consisting of a median and two lateral lobes. Otocysts closed, spherical sacs. Six pairs of nephridia opening on segments 5-10. One pair of oesophageal pouches, cylindrical, club-shaped. Diaphragmatic pouches large and finger-shaped. Body length about 25-40 mm.

*Remarks:* The species is widely distributed along the Japanese coast from Hokkaido to southern Japan and the Korean Archipelago.

#### Family Maldanidae

##### Genus *Praxillella* VERRILL

A slanting rimmed cephalic plate. Acicular ventral bristles in the first three setigerous segments. Several anteanal achaetous segments. Pygi-

dium funnel-shaped, bordered with cirri. Anal cone protruding. Ventral cirrus much larger than the others.

***Praxillella affinis* (M. SARS)**

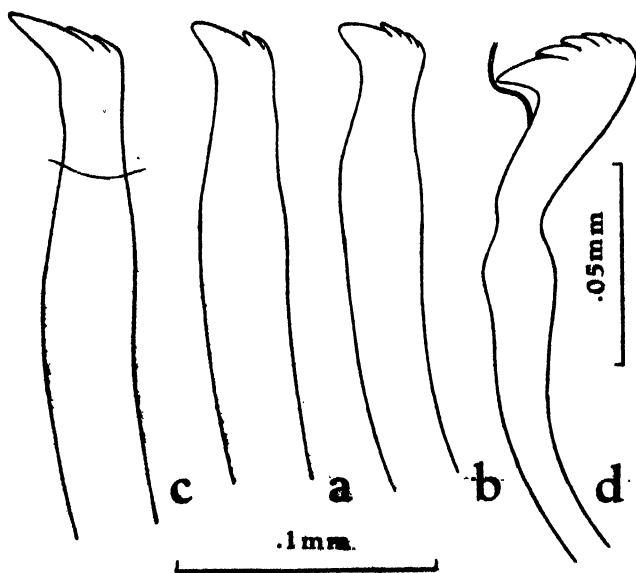
(Pl. II, Fig. E; Text-fig. 4)

*Praxillella affinis*: ARWIDSSÓN, 1906, p. 177, pl. 4, figs. 145-152, pl. 9, figs. 297-301, pl. 12, figs. 364-366.

*Clymene* (*Praxillella*) *affinis*: FAUVEL, 1927, p. 180, fig. 62, f.l.

**Occurrence**: Tako-Shima. Collected by dredging. Sp. No. H. 124.

**Specific characters**: There are 18 setigerous and 3 anteanal achaetous segments. Ocelli in 3 groups. The anterior point of the head is rather short, not ending in a filiform tip. Nuchal organ long. One to three ventral acicular spines in the first 3 setigerous segments. They bear 2-5



Text-fig. 4. *Praxillella affinis* (SARS). a, b, Hook from 1st neuropod; c, Hook from 3rd neuropod; d, Hook from 13th neuropod.

small teeth above a main fang. In the rest of body, uncini with 5-7 teeth. Dorsal capillary setae of 2 kinds. Pygidium fringed with 12-27 anal cirri, of which a median ventral one much longer. Glandular belts well developed from the 3rd to the 8th chaetigers. Nephridia in the 6th-9th chaetigers.

**Remarks**: Three complete specimens and several fragmental ones were



collected. The body length measures 26–58 mm. The anterior point of the head does not form a finger-like cephalic point as in *Praxillella gracilis*. There are 3 groups of the ocelli behind the cephalic portion. Anal cirri 14–16, with an exceedingly elongated slender ventral one. Uncini in anterior 3 setigerous segments, 1 or 2 in number. They are markedly open and bear 2–4 small teeth above a main fang. This boreal species is first recorded from Japan.

### Family Amphictenidae

#### Genus *Pectinaria* LAMARCK

Tentacular membrane fringed. Dorsal border smooth or crenate. Scapha separated from abdomen by constriction. Two pairs of branchiae. Uncini from the 4th setigerous segment, pectiniform, with teeth of different sizes. Dorsal bristles of two kinds; with slender smooth tips and with serrated tips. A single pair of cement glands. Three pairs of nephridia. Tube thin-walled, straight or curved.

#### *Pectinaria* (*Cistenides*) *hyperborea* MALMGREN

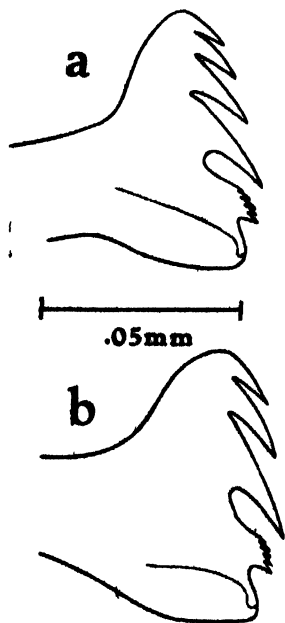
(Pl. II, Fig. F; Fig. 5)

*Pectinaria* (*Cistenides*) *hyperborea*: NILSSON, 1928, p. 31, fig. 9.

*Cistenides hyperborea*: MOORE, 1903, p. 479.

*Occurrence*: Onagawa Harbour. Collected by dredging. Sp. No. H. 109.

*Specific characters*: Tentacular membrane with 30–35 processes. Twelve to fifteen paleae on each side. Dorsal border of cephalic plate smooth. Seventeen setigerous and 12 uncinigerous segments. Dorsal bristles of two kinds; narrow, winged with delicate hirsute ends, and setae with curved tips, strongly denticulated on concave side. In profile uncini have 3–4 large teeth and 4–5 small ones above the basal process. Scapha longer than broad, with denticulated edges. Anal ligule broader than long, with a smooth edge and a small cirrus. Anal



Text-fig. 5. *Pectinaria* (*Cistenides*) *hyperborea* MALMGREN.  
a, b, Uncini.

hooks 6-9 on each side. Tube slightly curved, consisting of small brown sand grains.

**Remarks:** Two specimens rather large, measuring 48-50 mm were collected. They have 12-13 paleae. Tentacular membrane with 32-35 processes. Uncini have 3-4 large teeth above denticulated basal region. Anal hooks 7-8. NILSSON recorded the species from North Japan and MOORE from Sendai Bay. NILSSON considered that the Japanese form may be subspecies of the typical form. The Japanese specimen examined by him has fewer paleae (10-11), 4 large teeth in the uncini instead of 3 and fewer anal hooks, 5-7 instead of 6-9. These differences between the Japanese specimen examined by NILSSON and the typical form may be considered as varietal in character.

**Former localities in Japan:** Tuboruti, North Japan (NILSSON); Sendai Bay (MOORE).

### Family Terebellidae

#### Genus *Amphitrite* O. F. MÜLLER

Three, rarely two, pairs of branchiae, usually ramified, exceptionally cirriform and arising from a common base. There are 17-25 setigerous segments. Lateral lobes present in anterior region. Notopods from the 4th segment and neuropods from the 5th (2nd setigerous segment). Dorsal capillary setae with denticulated tips. Uncini set in biserial rows over a certain number of thoracic segments.

#### *Amphitrite ramosissima* MARENZELLER

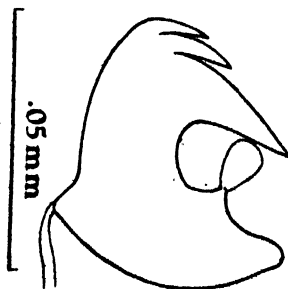
(Text-fig. 6)

*Amphitrite ramosissima*: MARENZELLER, 1885, p. 4, pl. 1, fig. 2.

*Neoamphitrite ramosissima*: HESSLE 1917.

**Occurrence:** Ishihama. Sp. No. H. 76.

**Specific characters:** Three pairs of ramified branchiae. No eyes. Lateral lobes on the 2nd and 3rd segments. About 13 gland-shields. The notopods begin on the 4th segment and occur in 17 pairs. Dorsal setae bordered with denticulated tips. Ventral uncini arranged in double rows from the 7th to 15th neuropods. Uncini with 3-4 rows of



Text-fig. 6. *Amphitrite ramosissima* MARENZELLER. Uncinus.

teeth above the main fang. Nephridial papillae from the 3rd to the 8th segments.

*Remarks:* The single specimen examined measures about 40 mm. Lateral lobes well developed. There are about 12-13 ventral gland shields. Nephridial papillae from the 3rd to the 11th segments. The present specimen corresponds well to MARENZELLER's species except for the number of the nephridial papillae, which MARENZELLER gave as occurring on the 3rd to 8th segment. *Amphitrite bifurcata* described by MOORE from Sendai Bay may be identical with the present species.

*Former localities in Japan:* Enoshima (MARENZELLER); Sagami, Sunosaki and Okinose (HESSLE).

### *Amphitrite cirrata* (O. F. MÜLLER)

*Amphitrite cirrata:* MALMGREN, 1865, p. 375, pl. 21, fig. 53; MOORE, 1903, p. 473; HESSLE, 1917, p. 185; FAUVEL, 1927, p. 251, fig. 86, i-o.

*Occurrence:* Onagawa Harbour. Sp. No. H. 75.

*Specific characters:* Three pairs of cirriform branchiae. No eyes. Lateral lobes on the 2nd, 3rd and 4th segments. About 12 gland shields. Seventeen setigerous thoracic segments. Dorsal capillary setae bordered with finely denticulated tips. Uncini set in double rows from the 7th to the 16th uncinigers. Nephridial papillae in 7 pairs, on the 3rd and the 6th-11th segments.

*Remarks:* A single small specimen. The species is widely distributed in the arctic and boreal regions.

*Former locality in Japan:* North Japan (MOORE).

### *Amphitrite rubra* (RISSEO)

(Pl. II, Fig. G)

*Amphitrite rubra:* FAUVEL, 1927, p. 249, fig. 85, h-i; 1936, p. 80.

*Amphitrite vigintipes:* MARENZELLER, 1884, p. 199, pl. 1, fig. 1.

*Neoamphitrite vigintipes:* HESSLE, 1917, p. 183.

*Occurrence:* Miyaga-Saki, sandy bottom. Sp. No. H. 77.

*Specific characters:* Three pairs of ramified branchiae. Lateral lobes on the 2nd, 3rd and 4th segments, of which the last pair is less developed and transversely elongated. Thoracic setigerous segments number 20-24. About 13 gland shields. Uncini arranged in double rows from the 7th to last thoracic segments. Dorsal capillary setae with serrated tips. Twelve to fourteen nephridial papillae.

**Remarks:** Three specimens were examined. The largest one measures about 100 mm exclusive of the tentacles. There are 20 pairs of notopods. Ventral gland shields 13 in number. The uncini begin on the 5th segment and are arranged in 2 rows from the 7th to the first abdominal neuropods. The nephridial papillae occur from the 3rd to the 15th segments (13 pairs). The number of the thoracic setigerous segments as well as that of nephridial papillae seems to be variable.

**Former localities in Japan:** Enoshima and Kagoshima (MARENZELLER); Misaki (HESSLE); Uchi-Ura, Wakayama Prefecture (FAUVEL).

### Genus *Polymnia* MALMGREN

Three pairs of ramified branchiae. Eye-spots numerous. Lateral lobes on anterior segments. The first notopods are on the 4th segment and first neuropods on the 5th. Dorsal capillary setae smooth at tips. Uncini with an elongated base, a lateral spur and denticles above the main fang. They are set in double rows over a certain number of segments.

### *Polymnia nebulosa* (MONTAGU)

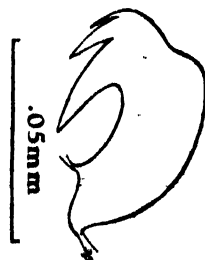
(Pl. II, Fig. H; Text-fig. 7)

*Polymnia nebulosa*: FAUVEL, 1927, p. 257, fig. 89, a-g; 1932, p. 224; MONRO, 1936, p. 180.

**Occurrence:** Onagawa Harbour and Shirokané-Zaki. Collected by dredging. Sp. No. H. 75 and H. 150.

**Specific characters:** Three pairs of ramified branchiae. Numerous eye-spots on the cephalic lobe. Lateral lobes on the 2nd, 3rd and 4th segments. Seventeen setigerous thoracic segments. Dorsal capillary setae smooth, narrowly bordered. They first appear on the 4th segment. Uncini with 2 large parallel teeth with a crest of 3-4 denticles above the main fang. Nephridial papillae from the 3rd to the 8th segments.

**Remarks:** A single specimen destitute of caudal region was collected. The uncini were not distinctly arranged in 2 rows. *Polymnia nesidensis* var. *japonica* recorded by MOORE from Sendai Bay is closely allied to the species. The species is first recorded from Japan.



Text-fig. 7. *Polymnia nebulosa* (MONTAGU).  
Uncinus.

Genus *Pista* MALMGREN

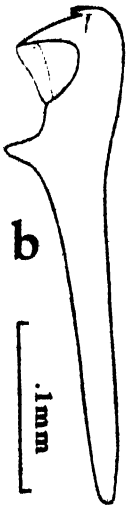
One to three pairs of bushy branchiae with a well-developed main stem. Lateral lobes in the anterior region. Distinct ventral gland shields. Notopods on the 4th segment and neuropods on the 5th. Dorsal capillary setae smooth. In the first few neuropods uncini with long shafts. In the rest of body uncini avicular, and arranged in double rows over a certain number of thoracic segments.

*Pista elongata* MOORE

(Text-fig. 8)



a



b

Text-fig. 8. *Pista elongata* MOORE. a, Uncinus from 1st neuropod; b, Uncinus from posterior neuropod.

*Pista elongata*: MOORE, 1909, p. 270, pl. 9, figs. 45-47; MONRO, 1933, p. 1068; BERKELEY, 1929, p. 5; 1936, p. 773.

*Pista maculata*: MARENZELLER, 1884, p. 204, pl. 1, fig. 5, a-d.

*Pista marenzelleri*: HESSLE, 1917, p. 157.

**Occurrence:** Onagawa Harbour. Collected by dredging. Sp. No. H. 117.

**Specific characters:** Three pairs of arborescent branchiae with a well developed main stem. Large lateral lobes on the 3rd segment. There are 17 setigerous thoracic chaetigers. Dorsal capillary setae narrowly bordered. Ventral uncini on the first neuropods have long shafts and a prominent anterior projection. The crest of uncini consists of a single transverse series of large teeth and the guard is large. Normal uncini of the rest of body have 2 or 3 rows of denticles above the main fang.

**Remarks:** A single specimen was collected. There are no lateral lobes on the 2nd segment. About 13 ventral gland shields. The shape of the uncini more closely coincided with that of MOORE's figure than that of MARENZELLER. As MONRO already mentioned the specific name of MARENZELLER's species, *Pista maculata*, first described from Japan, must be changed to *elongata* according to the rules governing nomenclature. According to BERKELEY the tube of this species composed of a chitinous material heavily beset with coarse particles of shells, sand, etc., similar that of *Lanice conchilaga*.

*Former localities in Japan* : Enoshima (MARENZELLER) ; Misaki (HESSLE).

Genus *Thelepus* LEUCKART

Two or three pairs of cirriform branchiae in a transverse series. Dorsal setae on a large number of segments. No lateral lobes in the anterior region. Notopods from the 3rd segment and neuropods from the 5th. Dorsal setae smooth ; uncini in a single row throughout the body.

*Thelepus setosus* (QUATREFAGES)

*Thelepus setosus* : FAUVEL, 1927, p. 273, fig. 95, a-h; 1936, p. 83.

*Thelepus japonicus* : MARENZELLER, 1884, p. 12, pl. 2, fig. 4.

*Occurrence* : Ommaé Inlet. Collected by dredging. Sp. No. H. 144.

*Specific characters* : Three pairs of filiform branchiae. Dorsal setae on the first 30-60 chaetigers. Abdominal region long, narrow. Uncinigerous tori form projecting, square pinnules in posterior abdominal region. Nephridial papillae on segments 4-7. Dorsal setae capillary of two kinds. Uncini with a transverse row of 2 or 3 large teeth above the main fang and above these 2 small teeth arranged in parallel. About 20 gland shields.

*Remarks* : An anterior fragment and several empty tubes cemented with shell particles and sand grains were collected. The species is widely distributed on Japanese coasts.

*Former localities in Japan* : Enoshima and Mazuru (Maizuru?) (MARENZELLER) ; Uchi-Ura, Wakayama Prefecture (FAUVEL).

Family Sabellidae

Genus *Potamilla* MALMGREN

Branchial lobes symmetrical, not spiral. Branchial filaments with or without eyes. No dorsal stylodes on the filaments. Winged setae of the first chaetiger in a tuft. Dorsal thoracic setae of two kinds ; winged capillary and spatulate. Thoracic tori with avicular uncini and pickaxe-shaped setae. In the abdomen dorsal avicular uncini and ventral winged setae.

*Potamilla reniformis* (O. F. MÜLLER)

*Potamilla reniformis* : FAUVEL, 1927, p. 309, fig. 107, a-l.

*Occurrence:* Izushima Harbour and Oûra Inlet. Collected by dredging. Sp. No. H. 92 and H. 93.

*Specific characters:* Body large. Two branchial lobes symmetrical. Nine to twelve thoracic setigerous segments. Ten to fifteen branchial filaments bearing 1 to 7-8 large dorsal eye-spots. Collar is four-lobed with two angular dorsal lobes. A conspicuous dorso-lateral incision above the first setae tuft. Setae of the first setigerous segment limbate. In the remaining thoracic region narrow winged capillary setae and oval spatulate setae with slender tips. Ventral tori with avicular uncini and pickaxe-shaped setae. In the abdomen dorsal avicular uncini and ventral bordered capillary setae. Tube horny, more or less incrustated with sand grains. The terminal portion of tube often rolled inwards.

*Remarks:* The fragmental, macerated specimen, together with several empty tubes, is referred to the species with some doubt. Tubes all rolled inwards at terminal portion. The species is here first recorded from Japan.

*Potamilla myriops* MARENZELLER

(Text-fig. 9)

*Potamilla myriops:* MARENZELLER, 1884, p. 211, pl. 3, fig. 2; OKUDA, 1934, p. 234, figs. 1 2; FAUVEL, 1936, p. 85.

*Occurrence:* Miyaga-Saki, in sandy beach. Sp. No. 77.

*Specific characters:* A large number of branchial filaments bearing 5 25 dorsal eye-spots arranged in a longitudinal row. Collar separated broadly on the dorsal portion. Collar setae narrowly double-winged. Other thoracic fascicles are composed of capillary setae and broad bladed spatulate setae. Thoracic tori having pickaxe-shaped setae and avicular uncini. In the abdomen dorsal uncini avicular and ventral setae limbate. Seven to fifteen thoracic setigerous segments. Tube rather thick and tough.

*Remarks:* The species commonly occurs along the northern Japanese coast.

*Former localities in Japan:* Sagami, Misaki, Sunosaki (JOHANSSON); Sêto (FAUVEL); Akkeshi, Muroran and Oshoro (OKUDA).



Text-fig. 9. *Potamilla myriops* MARENZELLER.  
Entire body.  $\times 1$ .

Genus *Sabella* LINNÉ

Branchiae not spiral, without dorsal stylods or subterminal eyes. The branchial filaments with or without eye spots. Collar lobed. Only winged setae in the first chaetiger. In the remaining thoracic chaetigers dorsal setae with wings of varying breadth and ventral avicular uncini and pickaxe-shaped setae. No spatulate setae. Abdominal dorsal uncini avicular and ventral setae winged.

*Sabella* sp.

*Occurrence*: Koshiki-Né. Collected by dredging. Sp. No. H. 66.

*Remarks*: A single specimen destitute of branchial filaments and posterior body region was collected. Nothing can be stated as to the character of the collar which is in fairly damaged condition. In the thorax ventral avicular and pickaxe-shaped setae; dorsal winged setae of two kinds, the one long, slender capillary and the other shorter with broader wing. On account of the absence of the branchial filaments and the damaged body an accurate specific identification is impossible.

## Family Serpulidae

Genus *Hydroides* GUNNERUS

Collar setae of bayonet shape with two conical processes at base of blade. Operculum funnel-shaped with a crown of horny spines arising from the centre. Uncini with a few coarse teeth, the lower one larger than the others. Thoracic setae winged, abdominal setae trumpet-shaped.

*Hydroides uncinata* (PHILIPPI)

(Text-fig. 10)

*Eupomatus uncinatus*: EHLERS, 1887, p. 285, pl. 58, figs. 6-11.

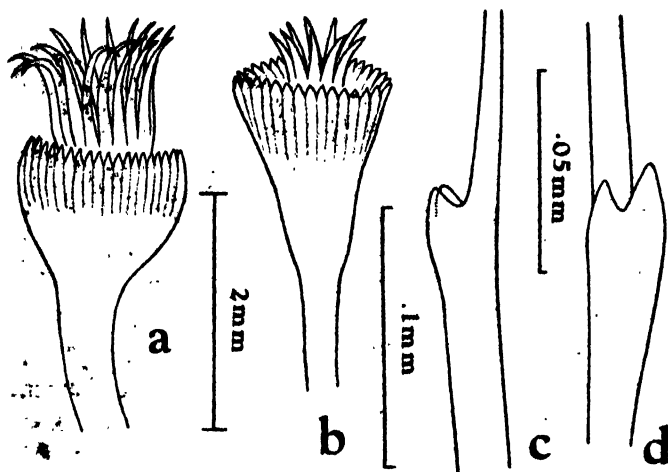
*Hydroides uncinata*: FAUVEL, 1927, p. 357, fig. 122, a-h.

*Occurrence*: Ishihama, on rocks. Sp. No. H. 88 and H. 136.

*Specific characters*: Opercular lower funnel with about 30 pointed spines, sometimes with a secondary short process at base; upper one with 8-12 tall spines all alike or accompanied by a different spine. Bayonet shaped collar setae with 2 short basal processes destitute of denticulations. Tube solitary or aggregated. They are cylindrical, wrinkled, often with dorsal carinae.



**Remarks:** A fair number of white cylindrical tubes, more or less coiled with two dorsal longitudinal ridges were collected. Two types of opercular funnel were found, viz., the one with upper crown bearing 16 long, tapered delicate simple spines pointing outwards and with serrated lower crown; and the other with upper crown having 8-9 short, strong spines slightly



Text-fig. 10. *Hydroides uncinata* (PHILIPPI). a, b, Two types of operculum; c, d, Setae from 1st chaetiger.

curved outwards and with denticulated lower crown. These spines of upper crown are all alike in both types. The shape of the opercular funnel of the former type is quite near that of EHLERS' figure. There are 15-17 branchial filaments on each side. The collar setae are of two kinds; slender capillary and bayonet-shaped. About 20-30 mm in body length. The species is now recorded from Japan for the first time.

#### Genus *Pomatoleios* PIXELL

Collar setae entirely absent. Uncini with fairly numerous teeth, the most anterior larger and gouged underneath. Abdominal setae trumpet-shaped with one side produced into a long spine. Operculum flat with winged pedicle. Tube with a flap over the entrance.

#### *Pomatoleios crosslandi* PIXELL

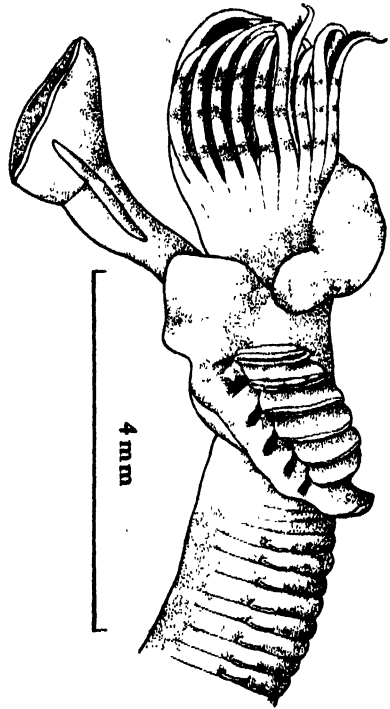
(Pl. II, Fig. I; Text-figs. 11-12)

*Pomatoleios crosslandi*: PIXELL, 1913, p. 85, pl. 9, fig. 10; FAUVEL, 1932, p. 243; MONRO, 1933, p. 508; DAY, 1934, p. 80.

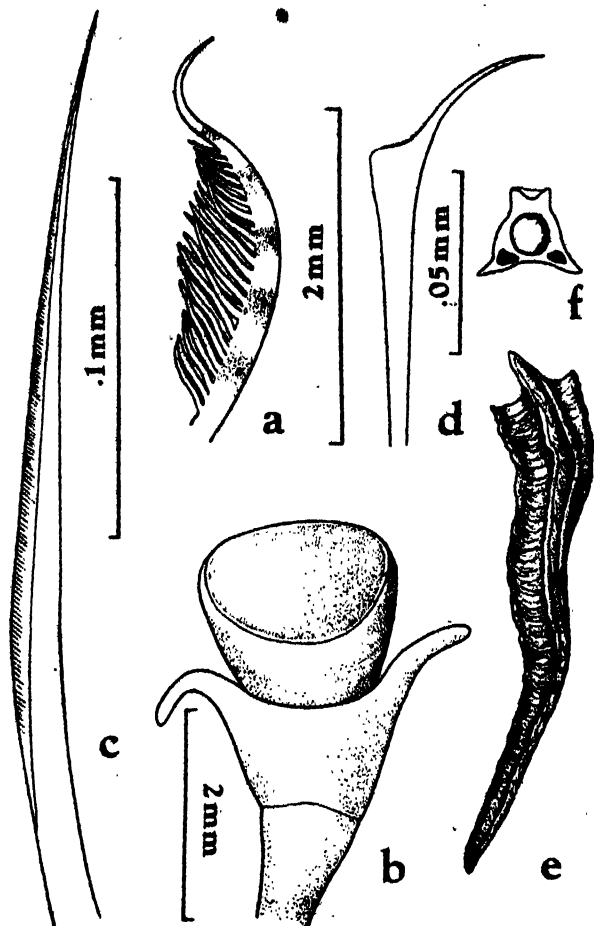
**Occurrence:** Ishihama, on rocks. Sp. No. H. 88.

**Specific characters:** All thoracic setae simple, striated. Branchial filaments with or without eye-spots. Uncini with 10 or 11 teeth in both the thoracic and abdominal regions. Collar membrane well developed. Branchiae with a high inter-branchial membrane and long bare terminal filaments. Opercular pedicle short with thick lateral wings. Tube, often blue coloured, having a flap over the entrance.

**Remarks:** Several tubes containing the worms together with that of *Hydroides uncinata* were collected. The present specimens have been compared with those collected from S to and Tomioka. They are about 15-25 mm in length. The branchial filaments, in 19-23 pairs, are well developed with long bare terminal portion. The eye-spots may be absent or sometimes present. The specimens from Tomioka are usually destitute of eye-spots, while those from S to and the present locality bear 3-5 distinct pairs of dark blue eye-spots arranged in a longitudinal row on each lateral side of the filaments. The branchial filaments are crossed by 3-5 blue or dark gray broad transverse bands. The collar membrane is well developed with a high flap-like ventral lobe. The flat operculum has a white calcareous plate. The opercular pedicle bears two lateral rod-like wings directed upwards. In all the specimens examined from various localities no collar setae can be found. The bristles and the uncini are as in usual cases. The thoracic uncini have 10 teeth above the gouge-like basal process and the setae are simple, striated, capillary. The tubes are blue in the outer as well as in the inner portion. They have long, triangular, pointed, prolonged dorsal processes beyond the entrance. Along the median dorsal surface there is a flattened more or less concave, broad ridge. The entire surface of the tube is graved by wavy



Text-fig. 11. *Pomatoleios crosslandi*  
PIXELL. Lateral view of anterior body.



Text-fig. 12. *Pomatoleios crosslandi* PIXELL. a, A branched filament; b, Operculum; c, Thoracic seta; d, Abdominal seta; e, Tube; f, Cross section of anterior portion of tube.

foldings. In cross section the anterior portion of the tube shows 2 short dorsal projections and a pair of basal sharp processes, and there are a large rounded hollow in the central portion and two lateral, small, more or less triangular ones, one on each basal processes. The genus is represented by a single species *crosslandi* originally described from the Red Sea by PIXELL. Afterwards FAUVEL recorded the species from Madras and both MONRO and DAY from South Africa. Japanese specimens have a rather large body and a greater number of branchial filaments. The occurrence of the eye-spots in some Japanese specimens may fall

within the variability of the species. As FAUVEL stated, the present writer also could not find a flap over the entrance of the tube. Although the species has not yet been recorded from Japan, it may be found fairly commonly along the southern Japanese coasts.

#### Genus *Salmacina* CLAPARÈDE

No operculum. Eyes present. Branchiae less in number (normally 8

filaments), with spatulate enlargements containing granular masses at their ends. Uncini pectinate with numerous small teeth. Hermaphrodite.

### *Salmacina dysteri* (HUXLEY)

(Pl. II, Fig. J)

*Salmacina dysteri*: FAUVEL, 1927, p. 377, fig. 129, c-k; PIXELL, 1913, p. 87.

**Occurrence:** Tsuka-Hama. Collected in a sandy beach. Sp. No. H. 112.

**Specific characters:** There are 7-9 setigerous thoracic segments. Branchiae in 2 lobes, each with 4 filaments with spatulate masses at their ends. Thoracic membrane well developed. Collar setae of two kinds; limbate setae and geniculate ones with numerous fine serrated processes at the base of blade. Other thoracic setae sickle-shaped or bladed forms. Tube calcareous, slender, filiform.

**Remarks:** A large mass of *Salmacina* tube containing a number of worms. This is the first record of the species from Japan.

### Genus *Spirorbis* DAUDIN

Body asymmetrical. Thoracic setigerous segments 3 or rarely 4. Operculum with a terminal calcareous plate having a peduncle without pinnules. Collar setae simple, bladed or with a fin-like extension at the base of blade. Abdominal ventral setae generally geniculate. Uncini with a free edge provided with numerous fine teeth. Calcareous tubes coiled in a dextral or a sinistral spiral.

### *Spirorbis* (*Dexiospira*) *nipponicus* OKUDA

*Spirorbis* (*Dexiospira*) *nipponicus*: OKUDA, 1934, p. 242, figs. 8-11.

**Occurrence:** Onagawa Harbour. Collected by dredging. Sp. No. H. 87.

**Specific characters:** Branchiae in 3 pairs. Collar setae with a simple serrated blade without fin-like expansion. Operculum with a blood-pouch which is cylindrical, dotted without longitudinal grated plates. Three thoracic setigerous segments. Uncini with fine marginal teeth arranged in 4-5 transverse rows, terminating in trifurcated basal processes. Tube dextral, keeled with 3 unequal dorsal carinae.

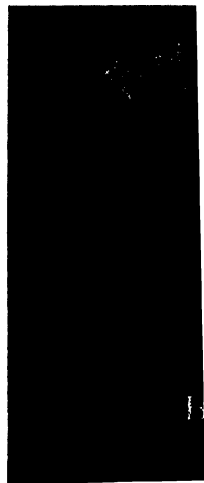
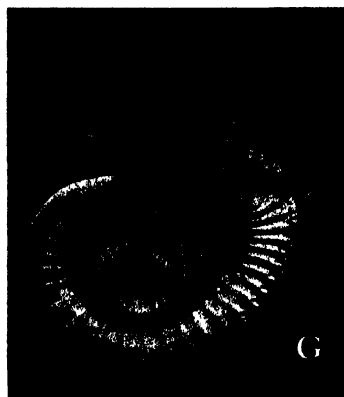
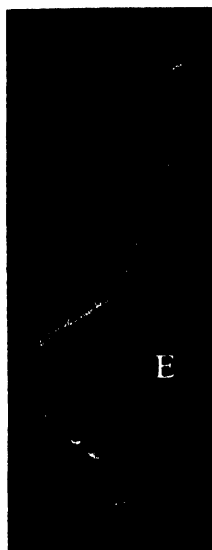
**Remarks:** The species closely allied to *Spirorbis foraminosus*, but only different from the latter in the uncini with the trifurcated basal processes

and in the operculum without a longitudinal grated plate. The species is commonly found fixed on sea-weeds.

*Former localities in Japan:* Akkeshi and Muroran.

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## EXPLANATION OF PLATE II.

- A. *Aricia fimbriata* MOORE.  $\times 2$ .
- B. *Audouinia comosa* (MARENZELLER).  $\times \frac{1}{2}$ .
- C. *Stylarioides plumosa* (O. F. MÜLLER).  $\times 1$ .
- D. *Brada villosa* (RATHKE).  $\times 2$ .
- E. *Prasillella affinis* (SARS).  $\times 2$ .
- F. *Pectinaria (Cistenides) hyperborea* MALMGREN.  $\times 1$ .
- G. *Amphitrite rubra* (RISBO).  $\times 1$ .
- H. *Polymnia nebulosa* (MONTAGU).  $\times 3$ .
- I. *Pomatoleios crosslandi* PIXELL. A mass of tube.  $\times 1$ .
- J. *Salmacina dysteri* (HUXLEY). A mass of tube.  $\times 1$ .





# SOME NOTES ON RELATIVE GROWTH, WITH SPECIAL REFERENCE TO THE GROWTH OF LIMPETS

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(With twelve figures)

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Postulating that the ratio of the specific rates of growth between the body and a part of it or between any two parts of it is constant, it is found that when the data of the relative growth are analysed, one or more breaking points in the curves are observed in the log/log plot of the two in many cases, e. g. in the limpets, *Patelloida grata*, *Patelloida conulus*, and *Patella vulgata*, in some arthropods (TEISSIER, 1935; PAULIAN, 1936; WEYMOUTH and MACKAY, 1936), and in some fishes (NEEDHAM, 1935). These points are usually considered as those marking off biological epochs of growth. But it is originally possible to recognize the breaking point as a critical one in physiological significance, only in the case where the postulate to the effect that the ratio of specific growth rates between the body and a part of it, or between two parts of it, is constant, is known to be true. In any examination of the postulate, the question arises as to whether it is true as we expected; consequently, it is necessary to study with accuracy the specific growth rate of a body or a part of it or of two parts separately. For these purposes, the growth of every part and of the body as a whole must be observed as a function of time or age. HAMAI (1935) has proposed an equation as one of those on which to base the formula of relative growth,  $y=ax'$ , in which the ratio of specific growth rates is constant. It may be, however, an approximate equation only fitting in with an early stage of growth, but not with the whole life.

This paper is intended to establish the general application of HAMAI's equation by re-analysing the data of ABE (1932) relating to the limpet, *Patelloida grata*, those of RUSSELL relating to *Patella vulgata* and the new data of the present writer<sup>1)</sup> relating to *Patelloida conulus*, and as a contribution to the study of the relative growth of these limpets. The present

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<sup>1)</sup> This was preliminarily reported as analysed by the method of application of the equation  $y=ax'$ , at the 12th Meeting of the Zoological Society of Japan (HAMAI, 1937).

writer wishes to express his sincere thanks to Prof. NOMURA for his cordial guidance and he is also indebted to Mr. S. NOMURA of the Saitô Hô-on Kai Museum for identification of the material species.

# EQUATIONS WITH REGARD TO RELATIVE GROWTH ON THE BASIS OF SPECIFIC RATE OF GROWTH

ABE (1932) measured the growth of *Petelloida grata* and gave the smoothed curves of it. He showed that two phases of growth, one of which is earlier than and the other is later than about the 6th or 7th year of the age, may be found by logarithmic plotting of both the shell length and the age. This fact indicates that the equation of specific growth rate,

$$R = \frac{1}{x} \frac{dx}{dt} = \alpha t^{-1} \quad (1)$$

viz. the equation set by HAMAI (1935), is not applicable throughout the life of this limpet. Nevertheless, it seems reasonable to consider the growth curve as a continuous one. And, it has been found that the specific rate of growth calculated by the formula,  $\frac{2(x_{n+1} - x_n)}{x_{n+1} + x_n}$ , where  $x_n$  is a dimension at time  $t_n$ ,  $x_{n+1}$  is at the next time  $t_{n+1}$ , which corresponds approximately to that at time  $\frac{1}{2}(t_{n+1} + t_n)$ , decreases in proportion to time in both logarithms. In these cases, the specific rates of growth were calculated from the probable smoothed curves in order to avoid the big deviations represented in the values of the measurements, especially in later periods of age. Heights and breadths are calculated from the ratios,  $B/L$ , and  $(B+L)/2H$ , where  $B$  is breadth,  $L$  length, and  $H$  height, as given in ABE's tables (Fig. 1). According to these facts, an extended form is derived from equation (1), i. e.

$$R = \frac{1}{x} \frac{dx}{dt} = \alpha t^{-m} \quad (2)$$

From the point of view that the specific rate of growth decreases monotonously with succeeding growth, LUDWIG (1929) has considered four cases of the simple law of growth, i. e.

- A)  $R = K(c - t)$
- B)  $R = K(c - x)$
- C)  $R = K/t$
- D)  $R = K/x$

$K$  and  $c$  being constants,  $t$  time and  $x$  the growing mass, and noted that there are no other simple laws of growth, but that it is possible for other

laws to conform to these four types. Equation (2) is a modification of (C) of these.  $\alpha$  in (2) is a specific rate of growth at  $t=1$ . In the earlier paper of the present writer (HAMAI, 1935),  $\alpha$  in equation (1) was defined

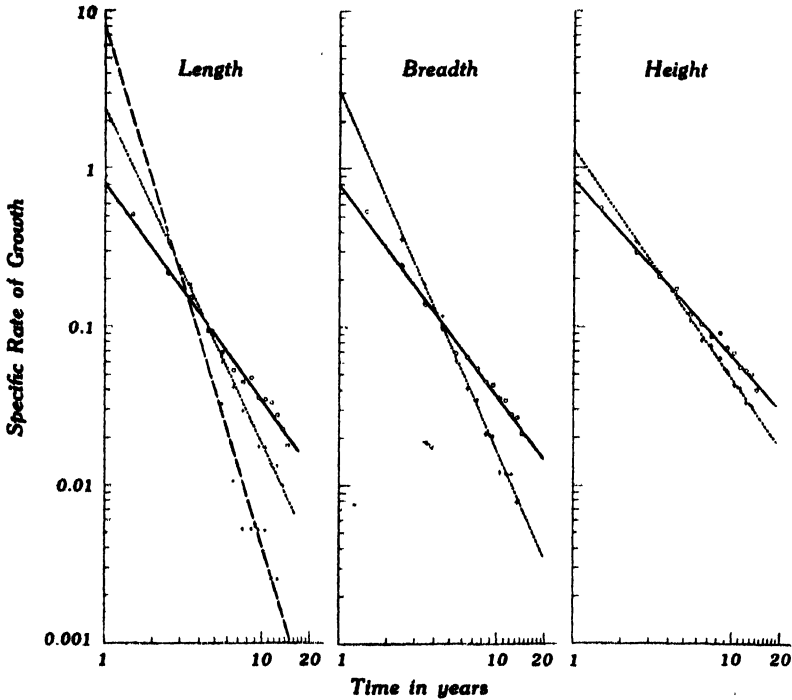


Fig. 1. Log/log plot of specific rates of growth relatively to time.  
 ○ dry localities at Mourajima, + damp localities at Mourajima, • dry localities at Ohshima.

as an index, which shows the decline of the specific growth rate in constant environmental conditions as regards its ecological significance. But in this case, by means of leading  $m$ , the significance of  $\alpha$  becomes difficult to define, for both  $\alpha$  and  $m$  ought to vary with different environments, and it is, therefore, probable that  $\alpha$  is merely taken as a specific rate of growth at  $t=1$ . In order to avoid confusion regarding the meaning of  $\alpha$ , equation (2) is to be re-written as follows;

$$R = \frac{1}{x} \frac{dx}{dt} = rt^{-m} \quad (3)$$

$$\text{or:} \quad x = Ae^{\frac{r}{1-m} t^{1-m}} \quad (4)$$

where  $e$  is the base of the natural logarithm, and  $A$  another constant. In equation (4), being  $m > 1$ , when  $t \rightarrow 0$ ,  $x \rightarrow 0$ , and when  $t \rightarrow \infty$ ,  $x = A$ ; therefore,  $A$  is the final dimension. Being  $m < 1$ , when  $t \rightarrow 0$ ,  $x \rightarrow A$ , and when  $t \rightarrow \infty$ ,  $x \rightarrow \infty$ ,  $A$  is, then, the initial dimension, and in this case the final dimension is infinite. Equation (4) has an inflexion point at  $(t = (m/r)^{1/(1-m)}, x = Ae^{m/(1-m)})$ , when  $m > 0$  and  $m \neq 1$ .

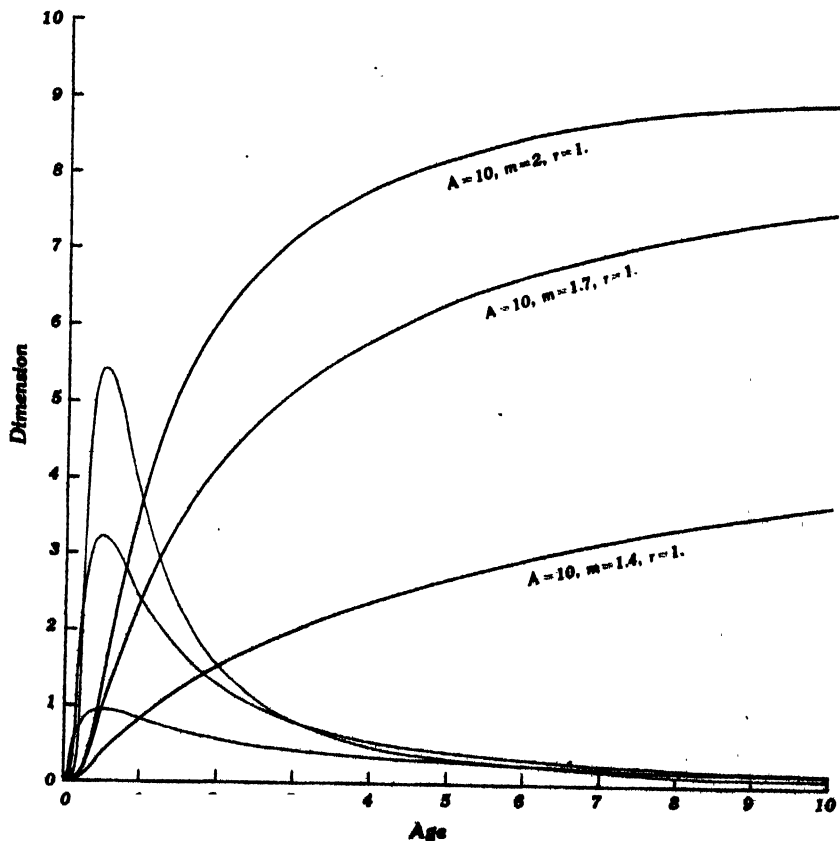


Fig. 2. Curves expressing equation (4) having different values for  $m$  and  $ds/dt$ -curves.

WEYMOUTH and his collaborators (1930 a, 1930 b) have analysed the growth of the mussels, *Siliqua patula* and *Cardium corbis*, on the basis of the specific rate of growth, and proposed an equation of growth —

$$R = r_0 e^{-rt} \quad (5)$$

They have proved that an inflexion point of growth curve in the clam, *Siliqua patula*, does not correspond with sexual maturity, and does not

indicate any physiological significance, and assumes the validity of the law of growth from MINOT (1908) to SCHMALHAUSEN (1929, 1931), i. e. that the intensity of growth due to an increasing proportion of inactive material in the organism and other causes is continually decreasing. SCHMALHAUSEN asserts the validity of his *law of progressive differentiation of the organism* which states that the relative amount of indifferent cell decreases in inverse proportion to time (age) in growing differentiated organisms: he maintains that the growth process, in which the specific rate of growth (his '*die wahre Wachstumsgeschwindigkeit*') decreases gradually in inversely proportional conditions to lapse of time, may be explained by the circumstances in which the law of progressive differentiation of the organism holds good. As regards SCHMALHAUSEN's law of growth, in many cases, the whole process of growth must be divided into several cycles; it is, however, only natural in such a case that equation (1) should theoretically hold good. No theoretical foundation exists in equation (1) or SCHMALHAUSEN's law of growth as pointed out by LUDWIG (1929), but it is empirically approximate. In the present case, of course, the inflexion point may have no physiological significance.

As equation (4) is applicable in two dimensions,  $x$  and  $y$ , there exist the following relation between  $y$  and  $x$ :

$$\left. \begin{aligned} \ln \frac{A_2}{x} &= k_0 (\ln \frac{A_1}{x})^\theta \\ k_0 &= \frac{r_2}{m_2 - 1} \left( \frac{m_1 - 1}{r_1} \right)^{\frac{1 - m_2}{1 - m_1}} \\ \theta &= \frac{1 - m_2}{1 - m_1} \end{aligned} \right\} \quad (6)$$

where the constants with the suffix 1 are related to  $x$ , and those with the suffix 2 to  $y$ , or in common logarithms

$$\log \frac{A_2}{y} = k_0 (\log e)^{1-\theta} (\log \frac{A_1}{x})^\theta \quad (7)$$

Equation (6) or (7) is a general form of the formula for relative growth. When  $\ln x$  is extremely smaller than  $\ln A_1$ ,

$$\begin{aligned} \ln \frac{A_2}{y} &= k_0 \ln A_1 (1 - \theta \frac{\ln x}{\ln A_1}) \\ \text{or} \quad y &= \frac{A_2}{A_1^{\theta}} x^{k_0 \theta} \end{aligned} \quad (8)$$

Equation (8) is the same form as the simple *allometrical*<sup>2)</sup> equation

<sup>2)</sup> The term *allometry* is proposed by HUXLEY and TEISSIER (1936) with the same meaning as heterogony.

(HUXLEY and TEISSIER, 1936) and this may be applicable during the period of smaller size in the early stages of the whole life. As it may be possible to assume approximately  $\theta=1$ , i. e.  $m_1=m_2$ , during a short interval, the simple allometrical formula may, accordingly, be also applicable in a short range of  $x$ . At any rate, the equation,  $y=ax^b$ , is a special case or an approximation. This is also derived from equation (5) at the time when it is proved that the constant  $p$  (decline constant) is the same for the two dimensions,  $x$  and  $y$ . The applicability of equation (4) and (7) to the limpets, *Patelloida grata*, *Patelloida conulus*, and *Patella vulgata*, is shown in Tables 1, 2, 4, and 6, and Figs. 5, 7, 10, 11, and 12.

In the study of relative growth, the ratio of the specific rate of growth comes into question. Its change follows the equation,

$$\frac{R_2}{R_1} = \frac{r_2}{r_1} t^{m_1 - m_2} \quad (9)$$

where  $R_1$ ,  $r_1$ , and  $m_1$  refer to the body or a part of it, as the standard, and  $R_2$ ,  $r_2$  and  $m_2$  to a part or another part. When  $m_1 > m_2$ , the ratio  $R_2/R_1$  increases with time parabolically; when  $m_1 < m_2$ , the ratio decreases with time hyperbolically; and when  $m_1 = m_2$ , it is constant during the whole life. That is,  $(m_1 - m_2)$  is an index indicating the conditions under which the specific growth rate of a part changes with time in comparison with that of the body or of the other part. Then, I propose the terms, *positive relative growth* for the case where  $m_1 - m_2 > 0$ , *negative relative growth* for the case where  $m_1 - m_2 < 0$ , and in accordance with the terminology of HUXLEY and TEISSIER (1936) *allometry* for the case where  $m_1 - m_2 \neq 0$ , and in addition *isometry* for the special case of  $r_1 = r_2$ . Intensity of relative growth is measured by the absolute magnitude of  $(m_1 - m_2)$ . This has a characteristic of intensity constant of relative growth. This is, therefore, termed *intensity index of relative growth*, representing simultaneously an equilibrium of relative growth, as expressed by the *equilibrium constant* of HUXLEY and TEISSIER (1936), which is the same as  $r_2/r_1$  in a special case of  $m_1 - m_2 = 0$ .

HUXLEY (1932) derived the simple allometrical equation from

$$\frac{dx}{dt} = \alpha xG \quad \text{and} \quad \frac{dy}{dt} = \beta yG \quad (10)$$

on the basis of three essential facts of growth, among which (1) growth is a process of self-multiplication of living substances, (2) the rate of self-multiplication slows down with increasing age, and (3) it is much affected by the external environment, e. g. by temperature and nutrition. He, then, considered that the two latter conditions affect all parts of the body

equally, so that it may be supposed that the growth rate of any particular organ is proportional simultaneously (a) to a specific constant characteristic of the organ in question, (b) to the size of the organ at any instant, and (c) to a general factor dependent on age and environment, which is the same for all parts of the body.

These assumptions are only generally admissible. The fact, however, that the rate of growth in each part is not exactly proportional to a general factor dependent on age and environment, has been found in the present investigation, i. e.  $G$  is different in each part of the body. Furthermore, another essential fact about growth is the addition of inactive material, e. g. in the shell-growth. This growth is, however, dependent on self-multiplication of the mantle tissues, if it is considered as a growth of the organism as a whole. DAVENPORT (1934) has criticized HUXLEY's theoretical justification of relative growth with reference to his studies on tadpoles, which indicate that their growth process is that of the imbibition of water for a considerable time, and he noted that the absolute amount of water added per day increased slightly during this period, but this growth is not because the protein molecules increase, but possibly because the lyophilic properties of these molecules become more efficient, perhaps just through their dispersal by the imbibed water: i. e. this is a qualitative rather than a quantitative change. DAVENPORT has further stated that it appears that HUXLEY's essential fact about growth is conceived too narrowly, and hence is not generally applicable.

HUXLEY has replied to the criticism of DAVENPORT viz. that growth involves increase in substances and that this increase, when the substance is alive, is fundamentally multiplicative and not additive. He conceives that the imbibition of water in tadpoles is only a special case, due to the fact that protoplasm is presumably highly concentrated in the egg, and that the first step in growth is mainly due to the imbibition of water. And he has cited the fact that water-content follows the law of allometry (TEISSIER, 1934). However, as stated also by BERNSTEIN (1934), in almost all cases of growth, self-multiplication is generally combined with addition.

HUXLEY has also noted that it might be expected, from certain experimental data, that the factor  $G$  would be a simple function of the deficiency in the size of the organism at any given time as compared with its final size; but this would not interfere with the validity of the more general formula. One cycle of growth obeys the logistic curve, e. g. as shown in *Meretrix meretrix* (HAMAI, 1935). The ratio of specific growth rates, then, probably varies during one cycle of growth but it may be



deemed cyclic, as explained later, although the variation is probably slight. A slight variation in ratio is found in *Meretrix meretrix* (Fig. 3). Such a slight variation as this is often ignored, and in this case the log/log

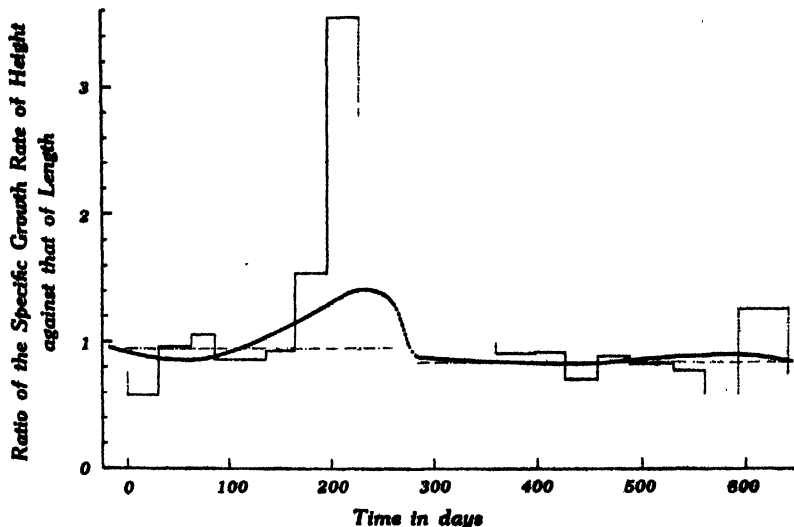


Fig. 3. Curves showing cyclic changes of the ratio between the specific growth rates of height and length in *Meretrix meretrix*. The stepped line is calculated from the actually observed values of each dimension. The thick line is calculated from the equations\*. The dash-dot line denotes average ratio according to the simple allometrical equation (HUXLEY's  $k$ ).

plot of two parts makes, approximately, a straight line, but it does not cover two or more cycles of growth, because the ratio of specific growth

\* In my earlier paper (1935), the constants of these equations are misprinted. They must be corrected as follows (*Italic figures*).

$$\text{Length, 2nd cycle: } \ln \frac{x-1.95}{3.20-x} = 0.0140(t-95)$$

$$\text{3rd cycle: } \ln \frac{x-3.18}{4.41-x} = 0.0120(t-450)$$

$$\text{Height, 2nd cycle: } \ln \frac{x-1.70}{2.72-x} = 0.0131(t-100)$$

$$\text{3rd cycle: } \ln \frac{x-2.70}{3.55-x} = 0.0118(t-450)$$

$$\text{Weight, 2nd cycle: } \ln \frac{x-2.00}{3.60-x} = 0.0140(t-111)$$

$$\text{3rd cycle: } \ln \frac{x-3.50}{10.50-x} = 0.0123(t-455)$$

The equations of the 2nd and 3rd cycles in height and length are used in this calculation.

rates increases or decreases from one cycle to the next (Fig. 4). Consequently, the simple allometrical equation has no continuity in one or

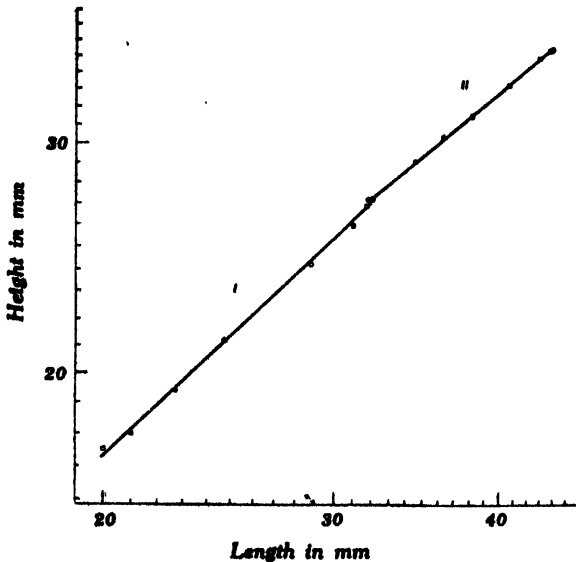


Fig. 4. Log/log plot of height against length in *Meretrix meretrix*.  
I:  $y=0.900x^{0.944}$ , II:  $y=1.032x^{0.985}$ .

more cycles of growth, and its validity is doubtful except as an approximation.

RICHARDS (1935) has proved that the actual constancy of the partition coefficient of HUXLEY [equilibrium constant in the new terminology (HUXLEY and TEISSIER, 1936)] may be examined only when the time component of the growth measurements is known. Re-analysing the data of JACKSON (1909), of TWITTY, and of MOMENT (1933), he called attention to the fact that in the analysis of growth the underlying reactions which may change with time must not be neglected, and that the use of a single average value of the partition coefficient is, in many cases, an unjustified over-simplification which prevents further elucidation of the growth of the organism. DAVENPORT (1934) has also concluded that "the suggestion has arisen that if the curves of growth of the two dimensions to be compared be plotted on ordinary graph paper,  $y$ , size, being plotted against  $x$ , time, the comparison of the tangents of their slopes at any age would give the relative growth ratio at that time: Or, the relative growth of the two curves may be expressed by comparing

their differential equations for any desired values of  $t$ ."

WEYMOUTH and MACKAY (1936) are of opinion that the method of analysis by means of the simple allometrical formula makes possible the detection of the changes of form which occur at certain physiological periods, notably that of attaining sexual maturity. It is a well-known fact that changes of form in the animal are correlated with sexual maturity, but, in a case in which these changes are monotonous and have no abrupt point in the alteration of the equilibrium, it is difficult to determine the size at which certain physiological periods occur, unless the physiological phenomena are observed together with the growth. In fact, DAWES and HUXLEY (1934) have said that it must be left undecided whether the temporary increases of the relative growth ratio of the nipper is associated with sexual maturity or not, and whether the very rapid decrease in the rate of growth of both claws, after attaining a certain size, is a phenomenon of post-maturity, — as seen in the secondary sex characters of *Gammarus*, — observing that the relative growth ratio of crusher and nipper changes generally diminishes with the growth period in the male of the pistol-crab, *Alpheus dentipes*. Recently, HUXLEY (1936) and DAY (1936) have observed that in the shore-crab, *Carcinus moenas*, the ratios of several parts, the abdominal limbs and the abdominal segments, gradually change from immaturity to maturity. According to their data, it appears that it is the alteration of the growth-centre that is important rather than the changes in the ratios themselves.

From the point of view of an assumption that all the organs in the same individual absorb the substances which sustain their maintenance and growth in the same environment of blood, and that, in the distribution of nourishment, they show an unequal need for this according to their nature and to their mass, certain hypotheses have been established by TEISSIER (1934, 1936) on the magnitude of this need; i. e. in the first approximation, it is possible to admit that the quantity of nourishment absorbed by an organ in a given time is proportional to the mass of this organ and to the total quantity of nourishment consumed during the same time; on the other hand it is possible to suppose that a definite fraction of the nourishment is disposed of in the growth of the organ. These hypotheses are written as follows,  $x$  being the mass of the organ,  $u$  the quantity of nourishment absorbed by this organ,  $U$  the quantity of nourishment available to the organism as a whole, and  $\lambda_1$  and  $\lambda_2$  being constants:

$$du = \lambda_1 x U dt \quad (11)$$

$$dx = \lambda_2 du \quad (12)$$

hence  $dx = \lambda_1 \lambda_2 x U dt$   
for other organs in the same manner:

$$dy = \mu_1 \mu_2 y U dt$$

$$\frac{1}{y} \frac{dy}{dt} = \frac{\mu_1 \mu_2}{\lambda_1 \lambda_2} \frac{1}{x} \frac{dx}{dt} \quad (13)$$

Then

$$y = Kx^\alpha, \quad \alpha = \frac{\mu_1 \mu_2}{\lambda_1 \lambda_2}, \quad K = \text{const.} \quad (14)$$

These hypotheses generally appear to be probable, but as regards (12) the increase of the mass of organ this increase is not only proportional to the quantity of nourishment absorbed by this organ but to the mass of the organ at that time or to age factors. GRAY (1929 a) has analysed the embryonic growth of the fish, *Salmo fario*, with regard to the yolk, and, considering that at any particular moment the yolk which is passing into the embryo is being subject to two distinct processes, i. e. (1) to maintain the respiratory and other katabolic processes of the tissues, (2) to form new tissues, he assumes that

$$\frac{dx}{dt} = kxu \quad (15)$$

and

$$-\frac{du}{dt} = \frac{dx}{dt} + k_1 x \quad (16)$$

where  $x$  is the dry weight of the embryo,  $u$  the amount of dry yolk available,  $k_1$  an average value of the amount of yolk required for maintaining the embryo, per gram of embryo, and  $k$  a constant. In fact, he has shown that the efficiency of development between the 50th and 80th day of incubation is constant, about 0.63 in *Salmo fario*; and furthermore attention is drawn by him to the fact that the efficiency of development of various types of animals is of the same order of magnitude (GRAY, 1927). From these assumptions, the growth relation of the embryo with the yolk is obtained as follows:

$$x = u_0 - u - k_2 \ln \frac{u_0 + k_2}{u + k_2}$$

where  $u_0$  is the dry weight of yolk in the newly fertilized egg, and  $k_2$  is a constant which alters with the temperature of incubation. It was shown that this equation holds good for the observed values.

From the facts above-mentioned, it may be possible to say that the analysis of GRAY is more reliable than that of TEISSIER in the point that the mass of the organ was considered, although the former's assumptions regarding the total growth of the embryo may not be applicable, as they do not stand to a single organ in the post-natal development.

If it is possible to suppose, empirically, that  $U$ , the quantity of nourish-

ment available in the organism as a whole, is expressed as a function of age, and, also, that there exists

$$dx = \lambda_2 f(t) du$$

with expression by the age factors instead of the mass factors, and further that

$$Uf(t) = \lambda_2 t^{-m},$$

then equations (3), (4), and (6) can be derived from these assumptions.

In recent years, BERTALANFFY (1934) has attempted to determine the metabolism-constant  $x$  (viz. exhaustion per unit of weight, per unit of time) from the growth equation, which is

$$\frac{dx}{dt} = \gamma o - x x$$

where  $x$  is the weight,  $o$  the surface area and  $\gamma$  the constructed mass in unit of weight per unit of surface area. The values of  $x$  calculated from the growth equation coincide with those determined by other physiological experiments in the case of mussels, crabs and fishes. But, as also stated by GRAY (1929 b), the conditions of growth are extremely complex, and it is difficult to express the growth rate of the organism and its organs in terms of rational units. Graphical treatment of the data underlying a typical growth curve is liable to result in errors of considerable magnitude, but the first approximation method is to be regarded as an empirical expression. The rate of growth of an organ is dependent on that of the other organs; it is, therefore, intrinsically difficult to express the behaviour of such a complex system.

#### PERIODICITY OF GROWTH

RUSSELL (1909) marked the limpet, *Patella vulgata*, on Dec. 31, 1907, in a bay near the Cloch, at Gourock in Scotland, and measured its growth from month to month during 1908. When his records were rearranged in the order of year-groups, an outline of its growth was obtained (Fig. 5). The growth during one year shows generally a sigmoid curve as found also in the case of *Meretrix meretrix*, and of *Nerita japonica* (SUZUKI, 1936), etc. The growth processes of insects and crustaceans are possibly recognized as special cases of the sigmoid curve proving that rapid growth occurs morphologically at the time of their moulting.

It has been noted by RUSSELL that the breeding season of *Patella vulgata* extends from July to December or to January, and by ORTON (1928 a) that it may extend from August to March at Plymouth in different seasons, and spawning may occur within this period. ORTON has

further noted that the maximum degree of spawning appears to occur about January-February and that shell-growth is general at the end of the breeding season. As mentioned above, the breeding season may extend so widely, that its limits may not be exactly determined in RUSSELL's

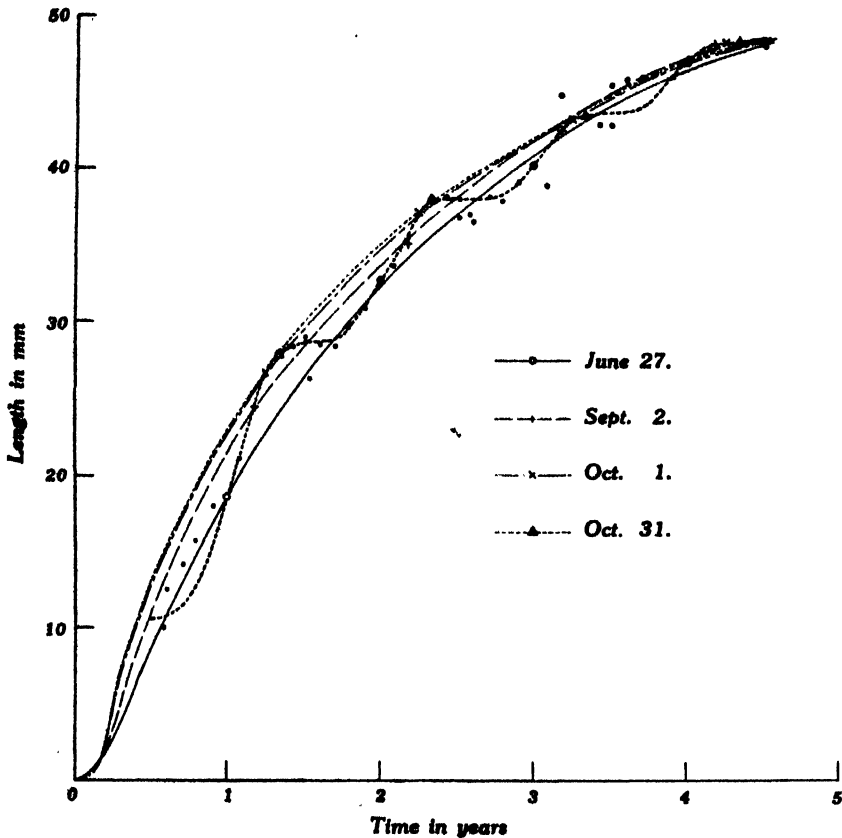


Fig. 5. Growth curves of *Patella vulgata*.

records; therefore, for convenience, June 27 was taken as the starting point for calculations in applying equation (4). The most probable measurements at the same date with the largest number of observations in his records were used in the calculation. Strictly speaking, although a certain time phase obeys the equation, another time phase may not exactly but only approximately follow it. It is, however, shown that the errors in applying the equation are practically very small in any time phase. The values of  $R$  obtained by this calculation in every time phase

does not coincide with the true specific rate of growth at that moment, but express the rate of annual change.

TABLE 1.  
*Patella vulgata*.

	Year group	June 27	f	Sept. 2	f	Oct. 1	f	Oct. 31	f
Observed and calculated (Italic figures) values	1st	18.6 <i>18.6</i>	33	24.5 <i>24.5</i>	30	26.7 <i>26.5</i>	30	27.9 <i>27.9</i>	24
	2nd	32.7 <i>32.5</i>	17	36.1 <i>35.4</i>	16	37.1 <i>36.8</i>	13	38.0 <i>37.7</i>	13
	3rd	40.3 <i>40.8</i>	4	42.5 <i>43.0</i>	6	43.2 <i>43.2</i>	6	43.4 <i>43.9</i>	6
	4th	47.0 <i>46.4</i>	1	48.1 <i>47.8</i>	3	48.2 <i>47.6</i>	3	48.3 <i>48.1</i>	3
Constants of equation (4)	<i>m</i>	1.637		1.583		1.550		1.545	
	<i>r</i>	0.990		0.824		0.751		0.735	
	<i>A</i>	88.1		88.1		88.1		88.1	
Mean of percentage deviation of observed values from calculated values		0.303±0.037		0.736±0.080		0.718±0.027		0.401±0.044	

The constants *m* and *r* vary little by little from one time phase to another, and they should oscillate theoretically with a definite amplitude, but the constant *A* ought to be always the same. The period of this oscillation is one year, and from the maximum to the minimum is the growth period, and the other period may be considered as the period of rest, during which the growth rate is very small or zero. A point touch-

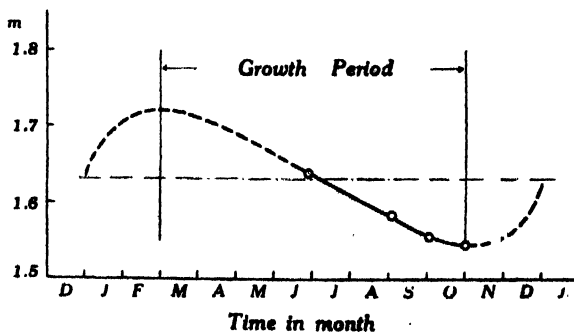


Fig. 6. Curve expressing the oscillation of the constant *m*. Points are observed values.

ing the axis of the oscillation, viz. a node of vibration during the growth period, is that showing the greatest rate of growth. The part of the oscillation during the rest period appears merely in the transition of time, and its variation has nothing to do with

the growth-rate. In practice, slips may more or less occur, as seen in the ratio of specific rates of growth (Fig. 3).

In the course of growth, the change of  $(m_1 - m_2)$  reveals that of the ratio of specific growth rate, as has been formerly stated. When  $m_1$  and  $m_2$  oscillate in such a way as mentioned above,  $(m_1 - m_2)$  must necessarily oscillate also. This fact indicates the change in the ratio between two specific rates of growth in different parts, and it has been already analysed in the case of *Meretrix meretrix* by the method of applying the allometrical equation, and further ascertained at present by the specific growth rates themselves (Fig. 3). The existence of an arrest of growth in midsummer in *Patella vulgata* has been detected by ORTON (1928 b). In this case, the existence of an arrest of growth ought also to appear during the periodical change of  $m$  or  $(m_1 - m_2)$ .

#### PROBLEMS OF VARIATION

A group of the same age has, to some extent, a variation in size. Now, the law of growth, as shown by equations (3) and (4), is one of those concerning the mean values of each age-group. When, therefore, the variation in size at the same age is considered, the applicability of equation (6) or (7) to all the variations may not be warranted, except for the mean values at each age of the group. The relation between two dimensions in an age-group may not always be parallel to the relation tracing the mean values at all ages of the groups. The proof of this fact will be given later. Anyhow, a suggestion is given by the fact, that the specimens of *Patelloida conulus*, larger than the mean value of the last age treated in the present investigation, slip out of the curve (Fig. 12).

#### GROWTH OF THE LIMPETS

##### I. *Patelloida grata*

The growth of this species has already been analysed by ABE (1932), and re-analysed by the present writer as shown in Table 2.

##### II. *Patelloida conulus*

*Patelloida conulus* is a small limpet and in the majority of cases, is found attached to the shell-surface of a gastropod, *Batillaria multiformis*. In one case only, it was observed, attached to the shell of *Littorina (Littorivaga) brevicula*, in Matusima Bay. 80 specimens were collected on



Sept. 11, 1935 at Hadutu-ga-Ura, Miyato Island in Matusima Bay. The largest among the specimens obtained was 7.7 mm in length and the smallest 2.7 mm, but the two largest specimens were omitted from statistical treatment because of the defacement of the apex in each case.

TABLE 2.  
*Patelloida grata*.

Habitat		m	r	A	Mean of % Deviation
Mourajima (dry localities)	Length	1.376	0.8021	49.61	$2.13 \pm 0.02$
	Breadth	1.326	0.7861	51.68	$2.20 \pm 0.06$
	Height	1.111	0.8523	3945	$3.41 \pm 0.07$
Mourajima (wet localities)	Length	2.123	2.397	34.19	$2.88 \pm 0.17$
	Breadth	2.276	3.109	27.88	$2.91 \pm 0.18$
	Height	1.442	1.314	40.36	$3.61 \pm 0.20$
Ohshima (dry localities)	Length	3.289	7.897	19.16	$3.92 \pm 0.04$

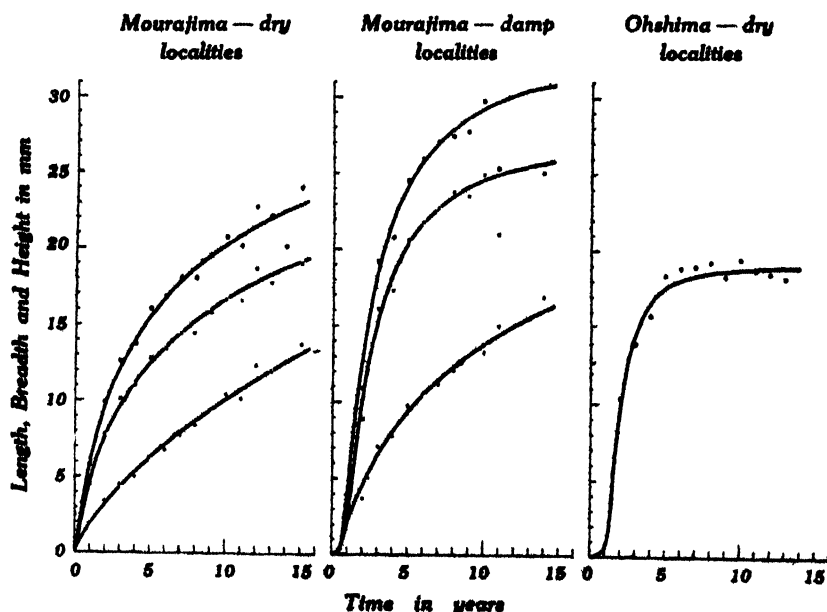


Fig. 7. Growth curves of *Patelloida grata*.  $\circ$  length,  $\times$  breadth,  $+$  height.

The differences between the largest and the smallest specimens were 4.1 mm in length, 3.3 mm in breadth and 5.9 mm in height. When all

the specimens were arranged, respectively, according to a difference in each case of 0.4 mm in length, 0.3 mm in breadth and 0.6 mm in height, three modes of frequency were recognized clearly in height, and slightly, in length and breadth at the points in the order of gradation (Fig. 8).

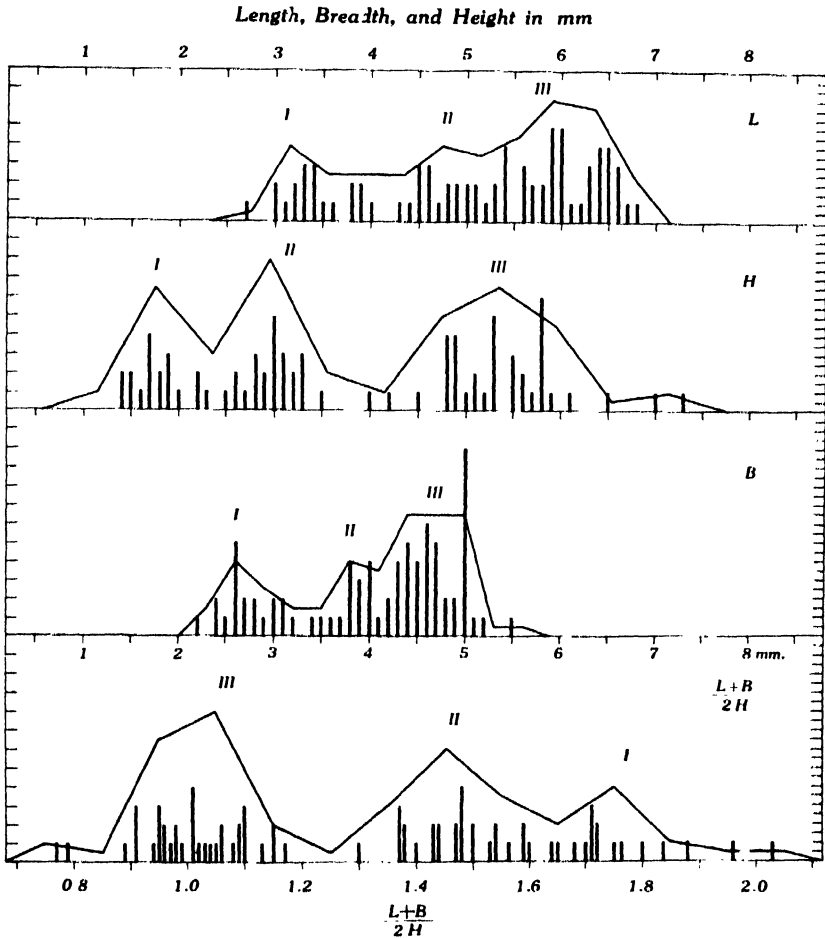


Fig. 8. Frequency polygons of size-groups in *Patelloida conulus*. I, II and III are deemed age-groups. One division of the frequency scale corresponds to one individual: the right scale for the polygons, and the left for each dimension actually measured (thick lines).

All the specimens therefore were divided into three groups, according to their respective mode. The number of specimens is not adapted to the

application of statistical treatment, nevertheless, the grouping is clear and each group follows the number of the rings of the growth line in the majority of specimens, i. e. group I has no ring, group II has one and group III has two; in the other specimens the ring was not clearly marked (Fig. 9). The results shown by the ratio  $(L+B)/2H$  which has been used by ORTON (1928 b) in his study of the growth of the limpet, *Patella vulgata*, as an index to show the variation of the shell-shape, and which has been observed to change from age to age by ABE (1932) in the case of *Patelloida grata*, are also clearly grouped in three. These three groups may, therefore, be understood as the age-groups. As the mean values of



Fig. 9. Photograph of *Patelloida conulus* of respective age.  $\times 2.3$ .

the respective age are unknown, an attempt was made to apply equation (4) to each dimension, taking the modes in length and the corresponding values of breadth and height as the representative values of respective age (Table 4 and Fig. 10).

TABLE 3.  
*Patelloida conulus*.

Range of length	f	aver. of length	aver. of breadth	aver. of height	aver. of $(L+B)/2H$
2.6 - 2.9	1	2.70	2.20	1.40	1.750
3.0 - 3.3	8	3.18	2.55	1.65	1.745
3.4 - 3.7	5	3.46	2.76	1.78	1.756
3.8 - 4.1	5	3.88	3.08	2.26	1.548
4.2 - 4.5	5	4.44	3.68	2.70	1.580
4.6 - 4.9	8	4.74	3.85	3.00	1.434
5.0 - 5.3	7	5.14	4.20	3.47	1.391
5.4 - 5.7	9	5.53	4.36	4.28	1.201
5.8 - 6.1	13	5.94	4.65	5.16	1.033
6.2 - 6.5	12	6.39	4.95	5.62	1.021
6.6 - 6.9	5	6.66	5.08	6.14	0.964

According to the constants calculated, it may be said that the height maintains the specific rate of the early stage of the period of growth almost without loss; on the other hand, the breadth loses most rapidly but has the highest rate among the three dimensions in the earliest age. The curve of height is a case of  $m < 1$ . The shell of group III is extraordinarily thick in comparison with that of the other groups.

### III. Relative growth

The transition of the specific rate of growth may be measured by the constants  $(m_1 - m_2)$  and  $r_2/r_1$ .  $r_2/r_1$  signifies an early state instead of the initial ratio which is unknown at present. From this point of view, it is found that, in the case of *Patelloida grata*, the breadth shows a slight negative relative growth relatively to length in damp localities but a very slight, positive or almost simple allometry, or isometry in dry localities, having a higher ratio in early stages in damp localities than in dry: height is positive relatively to length in both kinds of localities, but in damp places it is more intense, having a lower ratio at an earlier age than in dry places.

*Petelloida conulus* shows a more intense, relative growth compared

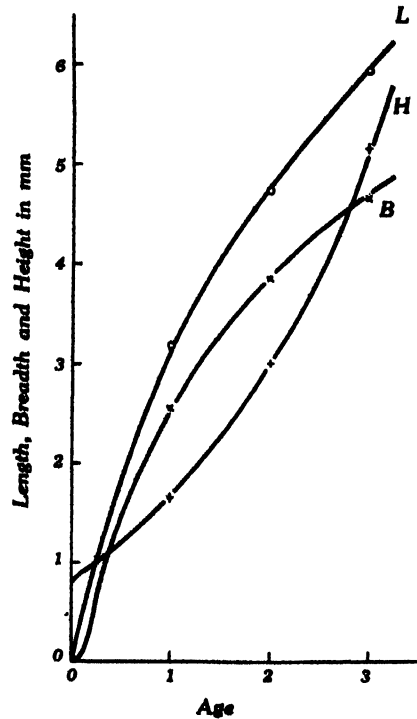


Fig. 10. Growth-curves of *Patelloida conulus*. L length, B breadth, H height.

TABLE 4.  
*Patelloida conulus*.

t	Length			Breadth			Height		
	obs.	cal.	dif.	obs.	cal.	dif.	obs.	cal.	dif.
1	3.18	3.14	0.04	2.55	2.50	0.05	1.65	1.67	-0.02
2	4.74	4.78	-0.04	3.85	3.88	-0.03	3.00	3.00	0.00
3	5.94	5.96	-0.02	4.65	4.70	-0.05	5.16	5.10	0.06
m	1.096			1.504			0.1802		
r	0.615			0.747			0.625		
A	1903			11.03			0.7800		

with *P. grata* in both dimensions, breadth and height, relatively to length. It can be clearly detected, at first sight of this species, that the height

TABLE 5.

Species	Relation	$m_1 - m_2$	$r_2/r_1$
<i>Patelloida grata</i> (Mourajima — damp localities)	<i>B—L</i>	-0.153	1.297
	<i>H—L</i>	0.681	0.548
<i>Patelloida grata</i> (Mourajima — dry localities)	<i>B—L</i>	0.050	0.980
	<i>H—L</i>	0.265	1.063
<i>Patelloida conulus</i>	<i>B—L</i>	-0.408	1.214
	<i>H—L</i>	0.916	1.017

TABLE 6.

*Relation of Two Dimensions.*

Species	Relation	$k$	$\theta$	Mean of % Deviation
<i>Patelloida grata</i> (Mourajima — wet localities)	<i>B—L</i>	1.163	1.136	0.72±0.07
	<i>H—L</i>	1.355	0.3933	2.76±0.11
<i>Patelloida grata</i> (Mourajima — dry localities)	<i>B—L</i>	1.118	0.8682	1.00±0.02
		1.097	0.8334	0.21±0.02
	<i>H—L</i>	3.422	0.2944	3.83±0.06
		3.444	0.2962	2.24±0.07
<i>Patelloida conulus</i>	<i>B—L</i>	$2.99 \times 10^{-3}$	5.25	1.09±0.07
	<i>H—L</i>	$-2.07 \times 10^3$	-8.54	2.41±0.11*

The Italic figures are those of calculation by means of the method of least squares with  $A$ -values in Table 1, applying the formula,

$$\log(\log \frac{A_2}{y}) = \log k + \theta \log(\log \frac{A_1}{x})$$

\*The mean of percentage deviation for the observed values except of the two largest in Table 3, which do not quite fit in the curve.

becomes markedly greater with the increase of age in comparison with the length (Fig. 9). The more vigorous relative growth may be considered as an adaptation to the environment, in which they live attached to a narrow surface of the shell of *Batillaria* in this respect differing markedly from the case of *Patelloida grata* which lives attached to rocks, and the relative growth of which is less vigorous than that of *P. conulus*. In the case of many other limpets, e. g. *Patelloida schrenckii*, *P. concinna*, *P. pygmaea*, *P. grata* and *Patella luchuana* and *Cellana nigrolineata*, *G.*

*torreuma*, *C. eucoemia*, etc., the shell is symmetrical to the antero-posterior axis, but in *Patelloida conulus* it is spirally twisted to the left; hence, in many other limpets, the plane containing the shell-margin is convex upwards or almost even against the antero-posterior axis, but, in this species, it is

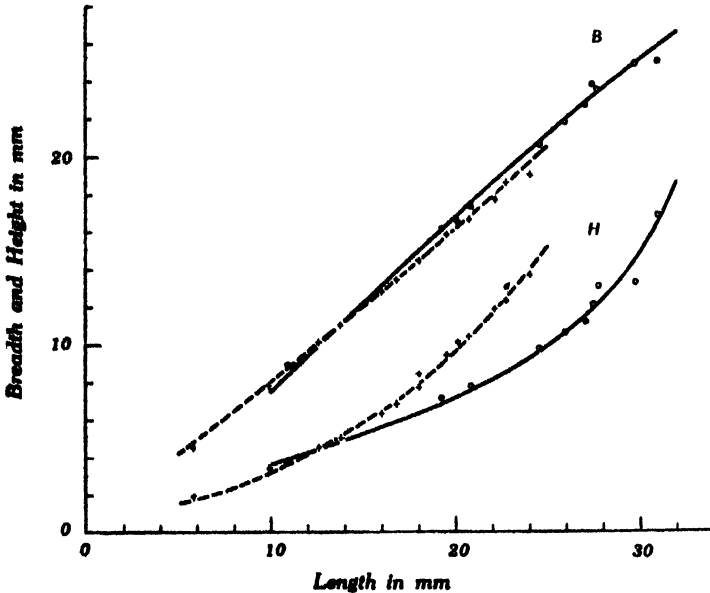


Fig. 11. Curves expressing the growth-relations in *Patelloida grata*. B breadth-length relation, H height-length relation.  $\circ$  damp localities at Mourajima, + dry localities at Mourajima.

convex upwards against an axis oblique to the antero-posterior (cf. the shell-margin in Fig. 9). The twist of the shell-margin of *P. conulus* seem to be correlated with the conical shell-surface of *Batillaria* as a result of the habit of the former to attach itself to the latter animal.

The environment, in which *P. conulus* grows, is a sandy beach between high and low water levels in Matusima Bay, so that it is, periodically subject to the effects of desiccation and of dampness. According to ORTON (1928 b) and ABE (1932), the shell-height of *Patella vulgata* and *Patelloida grata* is greater in a dry than in a damp habitat. The analysis of ABE's data, however, shows clearly, that the relative growth of height to length increases more rapidly in a damp than in a dry situation in the old ages. These facts are not contradictory, but give a rather more detailed account of the growth

It has been suggested by ORTON that the desiccation factors are most effective in causing shell-variations in different habitats, and that wave-action plays only a minor and secondary part in controlling shell-height. It has been further noted by ORTON (1929) that the behaviour of *Patella vulgata* is affected by oceanographical and climatic factors.

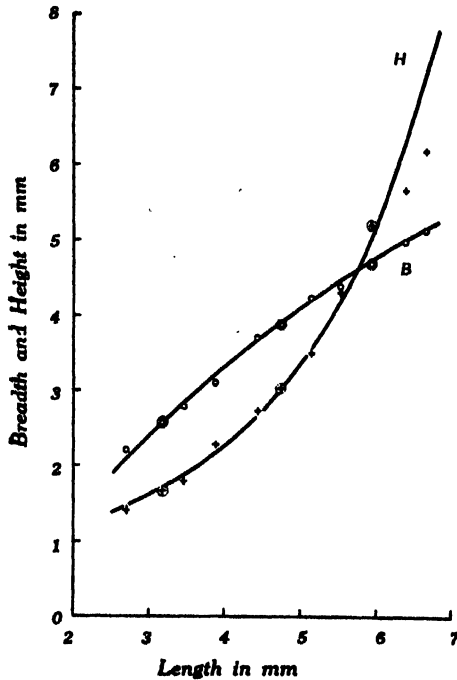


Fig. 12. Growth-relations in *Patelloida conulus*. B breadth-length relation, H height-length relation.

More evidence, concerning the effect of the level on which the animal live, is given by INO (1935) and by SUZUKI (1935) in the case of *Cellana toreuma*. They have stated that the higher the level from the datum level, the greater the height and the breadth. From this evidence, the conclusion follows that it is possible that desiccation or dampness affects the growth of limpets. INO conceives that there is also an effect of wave-action.

RUSSELL observed in *Patella vulgata* the changes of  $L/B$ ,  $L/H$ ,  $H/B$  and  $Ab/Af$ , where  $Af$  is the distance in a straight line between the apex and the anterior margin and  $Ab$  the distance between the apex and the

posterior margin, and found that the breadth and the height become greater relatively to the length, that  $Ab/Af$  becomes smaller from the smaller to the larger length group, and that the apex shifts gradually backwards. The difference of growth between the anterior and posterior margin is also true in the case of *Patelloida conulus*, i. e. the anterior margin grows rapidly, and the apex shifts gradually backwards (Fig. 9).

It has been noted by RUSSELL that the first sexual maturity is attained at a length of 20–25 mm and that there is marked fall in the rate of growth at 25 mm to half what it was at about 20 mm in the case of *Patella vulgata*. According to ORTON (1928 a), changes in shell-shape occur at 25–35 mm at Plymouth in *Patella*, and are coincident with the

change-over in sex-proportions. The sex phenomena in *Patelloida* are at present unknown, and the effects of these on the shell-growth are, accordingly, not evident, and call for further investigation, because it is impossible to deduce them from the growth-curve.

## SUMMARY

1) The growth of the limpets has been discussed on the basis of specific rate of growth.

2) The growth of the limpets obeys the empirical equation,

$$R = \frac{1}{x} \frac{dx}{dt} = rt^{-m} \quad \text{or} \quad x = Ae^{\frac{r}{1-m} t^{1-m}}$$

where  $x$  is a growing dimension, e. g. length, height, or breadth,  $r$  is a specific rate of growth at the time  $t=1$ ,  $m$  the decline constant of specific growth rate, and  $A$  the final size when  $m>1$  or the initial size when  $m<1$ ,  $e$  being the base of the natural logarithm.

3) The simple allometrical equation is derived from a special case of  $m=1$ .

4) The change in the ratio of specific growth rate is expressed by the formula,

$$\frac{R_2}{R_1} = \frac{r_2}{r_1} t^{m_1 - m_2},$$

in which  $(m_1 - m_2)$  is the intensity index of relative growth. This growth is positive when  $m_1 - m_2 > 0$ , and negative when  $m_1 - m_2 < 0$ , and allometrical when  $m_1 - m_2 = 0$ .

5) The relation between two parts obtained from the growth equations of each part, viz.

$$\ln \frac{A_2}{y} = k_0 \left( \ln \frac{A_1}{x} \right)^\theta, \quad \theta = \frac{1-m_2}{1-m_1}, \quad k_0 = \text{const.}$$

6) The periodicity of growth, viz. the change of  $m$  and  $(m_1 - m_2)$  in different time phases, has been discussed.

7) The relative growth of height relatively to length is positive in *Patelloida grata* and *Patelloida conulus*, and that of the latter is more intense than the former.

8) The relative growth of breadth relatively to length is negative in *Patelloida conulus* and in *Patelloida grata* which grows in a damp situation, slightly positive in *P. grata* in a dry situation, and that of *P. conulus* is more intense than that of *P. grata*.

9) The comparatively intense relative growth in *P. conulus* seems to be correlated with the environment, viz. the portion of the narrow surface



of the shell of *Batillaria*, to which it attaches itself.

10) In the case of *P. conulus*, the growth rate of the anterior margin is more intense than that of the posterior margin.

11) A relationship between the variation in size of the same age-group and the growth curve is suggested.

12) A relationship between growth-rate and sex-phenomena has been left for further investigation.

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# SYMBOLAE ITEOLOGICAE III

AUCTORE

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(Cum 6 tabulis et 9 figuris in textu)

(Opus acceptum d. XXVIII m. Maji a. MCMXXXVII)

- 18) × *Salix cremnophila*<sup>1)</sup> KIMURA *hyb. nov.* (Fig. 1, 2 & Tab. III, IV).  
= *Salix gracilistyla* MIQUEL × *S. japonica* THUNBERG.

*Frutex* ramis ramulisque primo declinatis deinde longe procumbentibus. *Ramuli* hornotini virides primo minute pilosi dein glabrescentes, basi circa insertionem barbati mox glabrescentes. novelli pubescentes; annotini graciles elongati glabri nitiduli castaneo-brunnei. *Rami* brunneo-virides deinde cinerascetes. *Cataphylla* steriliū ramulorum elliptica ad oblonga, utrinque obtusa, margine integerrima vel sat obsolete serrulata, supra viridia glaberrima nitentia, subtus dilute glauca nunc dense nunc sparse adpresse villosa, prima 12–13 × 4.5–5.5 mm, proxima 14–18 × 5.0–5.5 mm magna, superiora majora. *Folia recentissima* convoluta, brunnea, utrinque pilis acroscopicis adpressis villosa-sericea eundem in modum ut in *S. japonica*. *Folia adulta* chartacea, interstitiis 1.7–3.0 cm longis dissita, oblonga vel oblanceolato-oblonga,  $\frac{1}{2}$  fere sub apice vel medio latiora in superioribus, apice acuminata, basi margine convexo late vel subcuneatim acuta, margine tenuiter reflexa crenato-serrata, serraturis glandulis cartilagineis mucronulatis praeditis medio folii 2–4 pro 1 cm, sursum condensatis, deorsum laxantibus, ima basi integerrima, supra saturate viridia nitentia stomatifera, subtus dilute glauca, utrinque costa excepta glabrescentia, 10.5–12.5 cm longa 3.1–3.7 cm lata, 3.0–3.6-plo longiora quam latiora; costa pallide viridi, supra convexa pilis minutissimis directione variis pubescente, subtus vehementer prominente pilis adpressis acroscopicis pilosa; nervis primariis utroque latere 12–14, a costa sub angulis 40°–60° proficiscentibus, supra in sicco planis subtus acute prominentibus, arcuatis et sursum tenuioribus ante marginem flexuosis, secundariis subtus elevatis sat tenuioribus subparallelis crebris inter primarios transversis quasi modo *S. gracilistylae*, intermediis 0–2. *Folia inferiora* minora figura plerumque elliptico-oblanceolata, apice acuta vel in acumen breve producta, basi acuta, margine

<sup>1)</sup> Ε κρημνός, scopulus et πέδιλο, amo.

obsolete crenato-serrata, supra juxta nervos parcissime vel vix stomatifera, subtus caesia, utrinque praeter costas fere glabra, nervis secundariis et tertiis reticulum minutum formantibus, quae nota plus minus *S. japonicam*

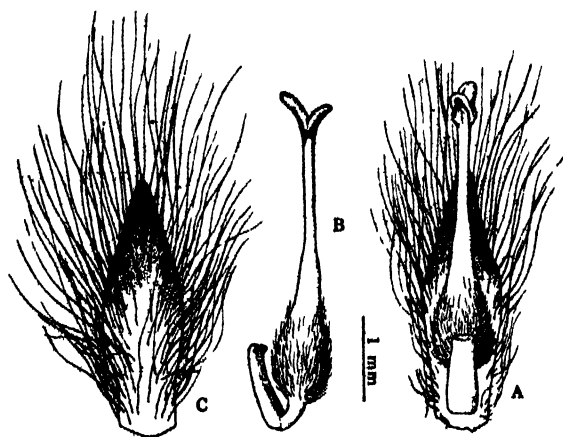


Fig. 1. *Salix crennophila* KIMURA  
A Flos ♀. B Pistillum cum glandula a latere.  
C Bracteola a dorso.

revocat. *Petoli* semiteretes, supra rubri pubescentes basin versus sulcati, subtus convexi virides glabri 1.2–1.7 cm longi. *Stipulae* semicordatae vel oblique ovatae, apice acutae, margine denticulatae, supra virides adpresse pilosae demum basi excepta fere glabrescentes, basi pauci-glandulosae, subtus dilute glaucae ab initio glabrae, in ramulis vernalibus minores, in vegetis 7–12 mm longae 3–5.5 mm latae. *Amenta* praecocia cylindrica curvula primo saepe nutantia, apicem versus paullatim angustata, apice obtusa, densiflora, villosa, foliato-pedunculata, 3–4 cm longa 6–8 mm crassa; pedunculis 3–5 mm longis pilis albis villosio-sericeis. *Cataphylla* pedunculi 2–4, elliptica, ovato-vel oblongo-elliptica vel oblonga, apice obtusa, basi sessilia vel subsessilia, margine integerrima, supra viridia glaberrima nudentia, subtus dilute glauca, adpresse villosio-sericea, 6×3, 9×3.5, 10×3.7, 13×4.5 mm etc. magna. *Bracteolae* elliptico-lanceolatae, apice angustato-acutae, dimidia superiore nigrae, medio pallidae, basi pallide flavo-virides, in expansione 2.7–3.0 mm longae 1.0–1.1 mm latae, utrinque pilis albis rectisque longe villosae. *Glandula* una ventralis oblonga truncata flavo-viridis 0.9–1.0 mm longa. *Ovaria* sub anthesi viridia e turgida basi anguste conica in stylum angustata, 1.5–1.6 mm longa, 0.7 mm crassa, dimidia inferiore parte sericea; stigmatibus glabris 0.3 mm longis; stylis filiformibus

1.4–1.5 mm longis, flavo-viridibus, ovaria longitudine fere aequantibus. *Stigmata* oblonga pallide flavo-viridia, commissuralia, divaricata, apice integra 0.6–0.7 mm longa.

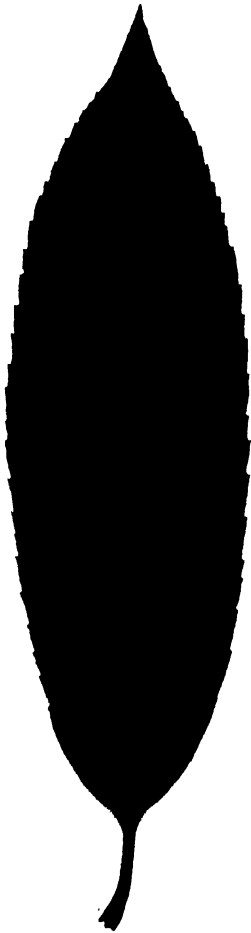


Fig. 2. *Salix cremnophila*  
KIMURA. Folium adultum nervaturam monstrans.  $\times 1$ .  
(Physiotypia)



Fig. 3. *Salix Isikawae*  
KIMURA. Folium adultum nervaturam monstrans.  $\times 1$ .  
(Physiotypia)

NOM. JAP. *Neko-siba-yanagi*, nom. nov.

HAB. JAPONIA. Honsyû.—Prov. **Sagami**: in ripa fl. Sakawagawa, Hukusawamura-Koiti, (A. KIMURA n. 2367 st. [typus] 16 Maio 1935 in Herb. A. KIMURA).—Prov. **Rikuzen**: Sendai, cult., (A. KIMURA n. 2688-

clonotypus [typus floris] ♀ fl. 10 Apr. 1937, fol. 23 Oct. 1936).

Stirpem originalem in praerupta ripa fluminis Sakawagawa crescentem inveni in viculo Hukasawamura-Koiti, haud procul ab oppidulo Yamakita. Surculi ex qua lecti sub n. 2688 nunc in saliceto meo Sendaiensi culti sunt. Existimo vero hanc salicem hybridam esse inter *S. gracilistylam* et *S. japonicam*, quae in classico loco vicinoque haud raro una inveniri possunt. Ab illa habet foliorum figuram nervaturam serraturam, costam subtus pilis acroscopicis obsitam, adultorum paginam supra stomatiferam, stipulas magnas semicordatas, amenta villosa praecocia, formam indumentum colorem bractearum et longitudinem maximam styli. Hanc autem potissime demonstrant rami tenues primo eleganter declinati, apex folii saepe sub-producto-acuminatus, amenta primo saltem non raro nutantia, nec non cataphylla pedunculi bene evoluta. Glandulae figura et indumentum ovarii tamen inter parentes fere media. A *S. gracilistylae* KIMURA, cui habitu et ceteris notis non absimilis est, differt: bracteolis extus minus dense villosis, ovarii dimidia superiore glabris et brevius stipitatis.

19) × *Salix Isikawae* KIMURA hyb. nov. (Fig. 3, 4 & Tab. V, VI).  
= ? *Salix sachalinensis* SCHMIDT × *S. alopochoa* KIMURA.<sup>1)</sup>

*Frutex* 2 m altus, ramis ramulisque erecto-patentibus vel adscendentibus. *Ramuli* graciles elongati nitiduli, hornotini autumno viridi-brunnei glabri, annotini primo vere fusco-purpurei glabri, novelli pilis crispulis minutissimis pubescentes, mox glabrescentes. Rami fusco-cinerei. *Gemmae* amentiferae oblongae obtusae fusco-rubrae pilis adpressis minutissimis puberulae vel fere glabrae circiter 7 mm longae vix 3 mm latae; foliiferae amentiferis aequiformes et concolores circiter 5 mm longae vix 2 mm latae. *Cataphylla* sterilium ramulorum elliptico-oblonga ad oblongo-oblancoolata, apice obtuse acuta, margine obsoletissime crenato-serrulata, sessilia, supra costa circa basin minutissime puberula excepta glaberrima, subtus adpresse albo-villosa, villis marginem superantibus, 7 × 2.5, 8 × 3, 11 × 3.5 mm etc. magna, prima et proxima lateralia. *Folia recentissima* utrinque tomentosa, e vernatione relaxata margine infero revoluta ad modum *S. sachalinensis* SCHMIDT. *Folia adulta* superiora chartacea interstitiis 1.3-2.8 cm longis dissita, lanceolata fere ad medium latiora, apice gradatim angustato acuta vel acuminata, basi margine leviter convexo acuta, 8-12 cm longa, 1.7-2.5 cm lata, 4.5-5-plo longiora quam latiora, margine crenato-serrulata vel -denticulata, dentibus in medio folii 3-5 pro 1 cm, apice folii crebris basi obsoletis,

<sup>1)</sup> *Salix alopochoa* KIMURA sp. nov. = *Salix vulpina* (non ANDERSSON) Auct. ex regionibus Kinki, Tyūgoku, Sikoku, Kynsyū. Ex *ἀλώπηξ*, vulpini-coloratus.

supra glabra nitidula saturate viridia impressinervata, infra glaucina parce pilosa, demum fere glabrata; costa supra purpurascente plana vel levissime convexa, basin versus minutissime pubescente, subtus pallide viridi prominente secus utrumque latus pilosa; nervis primariis supra impressis subtus prominentibus, utroque latere 13-17, leviter arcuatis a costa sub angulis  $50^{\circ}$ - $60^{\circ}$  divergentibus, secundariis tenuibus subtus elevatis inter primarios transversis subparallelis, intermediis 1-3 sub angulis latioribus exeuntibus. *Folia inferiora* ramulorum minora, oblanceolata, apice acuta, basi subcuneato-acuta, supra fere glabra, subtus sericea. *Petoli* supra purpureo-rubri leviter canaliculati pubescentes, subtus pallide virides fere glabri, ad 0.9 cm longi. *Stipulae* semi-cordatae, apice acuminatae, margine denticulatae, supra virides glabrae basi glanduliferae, subtus glaucinae parce pilosae vel glabrae, 4-10 mm longae 2.5-4 mm latae. *Amenta* coactanea oblongo-cylindrica densiflora villosa 1.7-2.8 cm longa 0.7 cm crassa, rachidibus pubescentibus, breviter foliato-pedunculata, pedunculis villosis ad 0.6 cm longis, cataphyllis 3-4 forma magnitudine indumentoque iis sterilium ramulorum simillimis suffulta. *Bracteolae* ellipticae, apice obtusissimae, dimidia superiore nigrae, inferiore pallidae circiter 1.5 mm longae 0.7 mm latae, utrinque albo-villosae. *Glandula* una ventralis lutea rectangularis vel ovato-rectangularis apice truncata 0.5-0.6 mm longa 0.4 mm lata. *Ovaria* ovato-conica sericea apice in stylum producta 1.5 mm longa 0.8 mm crassa, stipitibus sericeis ad 0.5 mm longis glandulam aequantibus. *Styli* obcompressi 0.7-1.0 mm longi pallide flavo-virides. *Stigmata* forma iis *S. sachalinensis* similia flavo-viridia ad 0.4 mm longa, apice emarginata vel bifida, laciniis oblongis ad 0.3 mm longis.

**HAB. JAPONIA.**—Prov. Settsu: monte Rokkosan, (E. ISIKAWA n. 2 ♀ fl. [typus] 23 Apr. 1935 in Herb. A. KIMURA, fol. 26 Jul. 1935).

Omnino tendet ad *S. sachalinensem*: figura cum nervatura folii, margine cuneato in juventute revoluta, forma et tomento ovarii, stylis evidenter elongatis. Bracteolae figura et color nec non brevitas glandulae forsitan

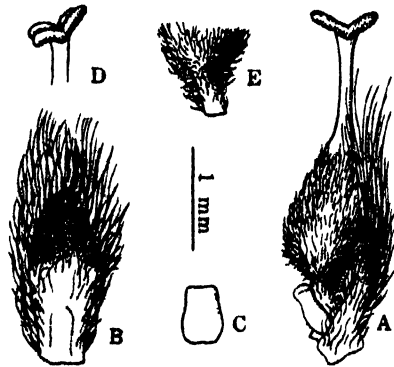


Fig 4 *Salix Isikawae* KIMURA

A Flos ♀ a latere B Bracteola a facie  
C Glandula ventralis D Stigmata  
E Stipes cum basi ovarii



mixturam cum *S. alopochroa* demonstrant.

20) × *Salix sirakawensis* KIMURA hyb. nov. (Fig. 5 & Tab. VII, VIII).  
= *Salix futura* SEEMEN × *S. integra* THUNBERG.

*Frutex* 2 m altus, ramis erecto-patentibus vel adscendentibus, cinereo-viridibus vel cinereis. *Ramuli* recti adscendentes elongati; hornotini 14–90 cm longi, autumno luteo- vel brunneo-virides, plus minusve nitentes; annotini gemmiferi primo vere lutescentes, luteo-brunnei, paullo nitentes. *Lignum* sub cortice vibiciferum. *Gemmae* amentiferae pulchre rubrae, glaberrimae, anguste ovatae, sursum acutae, ad summum obtusae, latere paullo vel vix carinatae, ventre moderatim dorso valde convexae, 9–11 mm longae, 4.5 mm latae; foliiferae rubrae, sat minores, ovatae, apice obtusae, ramulis adpressae, 3–4 mm longae, 2–2.5 mm latae. *Cataphylla* sterilium ramulorum viridia, elliptica ad oblonga, utrinque obtusissima, sessilia, integerrima, supra glabra subtus adpresse albo-sericea, 8–10 mm longa et 4 mm vel ultra lata, infima et proxima lateraliter disposita. *Folia recentissima* sub vernatione convoluta et utrinque pilis curvis minutissimis obsita, e vernatione relaxata pulchre rubra mox glabrescentia. *Folia adulta*

chartacea, interstitiis 0.8–4 cm longis dissita, oblonga, lanceolato-oblonga, nonnumquam oblanceolato-oblonga, lateribus plerumque fere parallelis, apice acuta vel breviter acuminato-acuta, basi cordata, margine crenato-serrulata, serraturis in medio folii 4–6, supra sat viridia nitidula, impressinervata, subtus intense glauca, utrinque (costa supra basi pulverulento-puberula excepta) glaberrima, 8.5–12.0 cm longa, 2.4–3.5 cm lata, 2.7–4-plo longiora quam latiora; costa pallida supra convexa, infra valde prominente; nervis primariis utroque latere 14–20, a costa sub angulis 50°–70° divergentibus, supra impressis infra prominentibus, leviter arcuatis, ante marginem plus minusve flexuosis et nonnumquam furcatis; secundariis tenui-

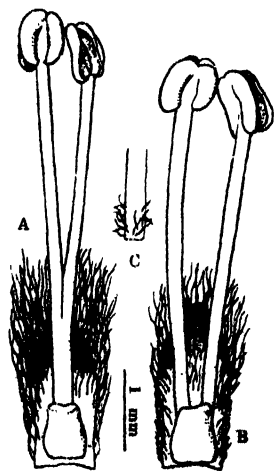


Fig. 5. *Salix sirakawensis* KIMURA.

A Flos ♂ filamentis inferne connatis.

B Flos ♂ filamentis liberis.

C Basis filamentorum connatorum.

bus supra impressis infra elevatis, inter primarios transversis flexuosisque; intermediis 1–3. *Petioles* 0.6–1.0 cm longi supra canaliculati minutissime

pubescentes, infra convexi glaberrimi. *Stipulae* in ramulis vegetis evolutae, oblique ovatae vel semilunatae, apice obtusae, margine externo obscure crenato-serrulatae, subtus glaucae, magnitudine sat variae, 2.5–8 mm longae, 1.7–4.4 mm latae. *Amenta* ♂ praecocia, densiflora, oblongo-cylindrica, apice rotundata basi subsessilia vel breviter foliato-pedunculata, 2.2–3.3 cm longa, 1.1–1.3 cm crassa, rhachidibus pubescentibus. Pedunculi villosi ad 3 mm longi, foliolis 3–7, oblongis, ellipticis, ovatis, apice obtusissimis ad obtusis, basi sessilibus, margine integerrimis, supra glabris infra albo-villoso-sericeis, pilis marginem superantibus, 6–9 mm longis 2.5–3.5 mm latis, superioribus minoribus. *Bracteolae* ovato-ellipticae vel elliptico-oblongae, apice obtusissimae, superne nigrescentes, medio rubicundae, basi pallidae, utrinque albo-villosae, circiter 2.0 mm longae et 1.0 mm latae. *Glandula* una ventralis lutea ovato-rectangularis apice truncata 0.5–0.6 mm longa et 0.4–0.6 mm lata. *Stamina* 2, filamentis 4–5 mm longis, ima basi paucipilosis, e basi ad medium usque vel altius connatis, cruribus sub angulo acutissimo divergentibus, nonnumquam toto liberis. *Antherae* ovatae circiter 0.8 mm longae 0.6 mm latae, ante anthesin rubrae, polline explosio fuscescentes.

NOM. JAP. *Kosekiyanagi*, nom. nov.

HAB. JAPONIA. Honsyû.—Prov. Iwaki: Kosekimura, (A. KIMURA n. 875 ♂ fl. [typus] 23 Apr. 1934 in Herb. A. KIMURA, fol. 7 Sept. 1934, 21 Oct. 1934, 15 Sept. 1935).

form. *pubescens* KIMURA form. nov.

A typo foliis adultis subtus in costa petioloque minute pubescentibus recedit.

NOM. JAP. *Sinanoyanagi* HISAUCHI in Jour. Jap. Bot. II. p. 50 (1919).

HAB. JAPONIA. Honsyû.—Prov. Sinano: Noziri, (K. HISAUCHI n. 1722 st. [typus] 12 Aug. 1936 in Herb. A. KIMURA); Izunahara, (K. HISAUCHI st. 5 Aug. 1918).

Habitu et characteribus inter *S. futuram* SEEMEN et *S. integram* THUNBERG ambigit forsitan ex iis hybrida. Ab illa habet lignum sub cortice vibiciferum, cataphylla subtus dense sericea, folia recentissima crispo-puberula, adulta supra impressa infra prominenter nervata; a *S.* autem *integra* figuram et grabritatem foliorum, antheras primo rubras, filamenta staminum saepissime alte connata. Magnitudo foliorum atque gemmarum inter parentes fere media, color autem gemmarum ad *S. integram* appropinquat.

21). *Salix Kimurana* (MIYABE & TATEWAKI) MIYABE & TATEWAKI in Trans. Sapporo Nat. Hist. Soc. XIV. p. 255 (1936); in Proc. Imp. Acad.

Tokyo XHI. p. 26 (1937).

Syn. *Salix berberifolia* PALLAS var. *Kimurana* MIYABE & TATEWAKI in Trans. Sapporo Nat. Hist. Soc. XIV. p. 84 (1935).

Fig. nostrae 6, 7, 8.—*Fruticulus* circiter 7 cm altus (ex MIYABE & TATEWAKI), trunco subterraneo brunneo 4 mm vel ultra in diametro. Rami procumbentes, annotini luteo-virides vel luteo-brunnei, laeves glaberrimi 7.0–10.0 cm longi, 1.3–2.5 mm crassi; veteriores brunnei glaberrimi laeves quasi vernicosi, circiter 4 mm crassi. *Ramuli* hornotini in sicco flavo-virides laeves sub lente parce puberuli 3.4–6.7 cm longi 1.0–1.4 mm diametrientes. *Gemmae* ovato-globosae glaberrimae lutescentes vel luteo-brunneae 2.5–4.0 mm longae. *Folia* coriacea rigidiuscula, supra non stomatifera, exsucca hieme non decidua, demum marcescenti-persistentia; adulta superiora interstitiis 4–17 mm longis dissita, obovata, apice rotundata vel late acuta, basi acuta, margine grosse et acute dentato-serrata, serraturis inaequalibus rectis vel incurvis vel patentibus, rigidis, in medio folii 1.7–2.0 mm inter se remotis, supra viridia subtus paullo pallidiora, utrinque glaberrima et subnitentia, 20–32 mm longa, 11–18.5 mm lata, 1.6–1.9-plo longiora quam latiora; costa in sicco supra fere plana infra prominente, nervis primariis utroque latere 5–7, inferioribus leviter curvulis vel fere rectis, superioribus arcuatis, sub angulis 25°–40° divergentibus, utrinque elevatis, secundariis plus minus flexuosis inter primarios transversis; nervis intermediis brevibus raro evolutis. *Folia* inferiora obovata vel anguste obovata, minora, minus grosse serrata. *Petoli* straminei supra profunde canaliculati infra teretes glaberrimi 4–7 mm longi. *Stipulae* bene evolutae ellipticae vel elliptico-lanceolatae vel lanceolatae, apice acutae, margine glanduloso-denticulatae, uncostatae, supra sat virides, infra paullo pallidiores, utrinque glaberrimae 3.0–7.0 mm longae 1.5–2 mm latae. *Cataphylla* infima lateralialia vel oblique lateralialia, obovata vel anguste obovata minute denticulato-serrulata, utrinque glaberrima 8–11 mm longa 3.6–4.5 mm lata, petiolis ad 3.5 mm longis. *Amenta* tantum fructifera visa cylindrica foliato-pedunculata, basi perula gemmae persistente, 2.4–3.8 cm longa, circiter 8 mm crassa, fructibus non condensatis, rhachidibus pubescentibus per totam fere longitudinem visibilibus; pedunculi hirsuto-pubescentes 12–19 mm longi, foliolis 2–3 cataphyllis sterilibus ramulorum similibus obovatis vel anguste obovatis, apice obtusis basi acutis, margine serrulatis, supra sat viridibus infra paullo pallidioribus, utrinque glaberrimis vel subtus inferne ad costas solum pilosis, 7–15 mm longis 3.5–5 mm latis, petiolis ad 3.5 mm longis. *Bracteolae* obovatae apice rotundatae interdum leviter emarginatae, dimidia superiore parte fuscescentes inferiore pallidae, utrinque



Fig. 6. *Salix Kimurana* MIYABE & TATEWAKI. Typus.  $\times 1$ .

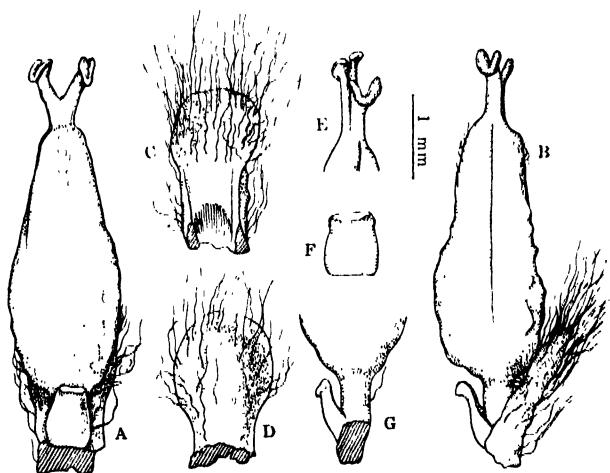


Fig. 7. *Salix Kimurana* MIYABE & TATEWAKI.

*A* Flos ♀. *B* Idem a latere. *C* Bracteola a facie. *D* Eadem a dorso. *E* Stigmata cum stylo et summa parte ovarii. *F* Glandula ventralis a facie. *G* Basis ovarii cum stipite et glandula ventrali a latere.

hirsutae (intus basi glabrae) 1.7–1.8 mm longae 0.8–1.2 mm latae. *Glandula* una ventralis ovata vel ovato-oblonga apice truncata 0.7–0.9 mm longa



Fig. 8. *Salix Kimurana* MIYABE & TATEWAKI KAWASHIMA n. 1.

0.4–0.6 mm lata. *Capsulae* ex ovata basi elongato-conicae, superne latere dorsali et ventrali minutissime crispo-pilosae, ceterum glabrae 3–4.5 mm longae; stipitibus circiter 0.6 mm longis, superne dorsali latere crispo-

pilosis. Styli obcompressi 0.6–0.9 mm longi apice bifidi, laciniis 0.2–0.3 mm longis. Stigmata bifida, laciniis 0.2–0.3 mm longis.

NOM. JAP. *Karahuto-megiyonagi* MIYABE & TATEWAKI in Trans. Sapporo Nat. Hist. Soc. XIV. p. 84 (1935).

HAB. JAPONIA. Sachalin austr.—Distr. Sikka: the upper Rukutama, (M. KAWASHIMA [typus] 2 Aug. 1935 in Herb. Facult. Agr. Imp. Hokkaido Univ.; n. 1, 16 Jul. 1935).

A *S. berberifolia* PALLAS, cui valde affinis est, foliis majoribus supra non stomatiferis, stipulis multo majoribus, amentis in fructu longioribus, capsulis superne latere dorsali ventralique crispo-pilosis facilliter distinguenda.—Plurima specimina *Salicis berberifoliae* PALLAS misit libenter mihi ut compararem ill. Dr. G. KOIDZUMI Universitatis Imperialis Kyotensis Professor, cui hoc loco gratias ago.

22) *Salix gracilistyla* MIQUEL in Ann. Mus. Bot. Lugd.-Bat. III. p. 26 (1867); Prol. Fl. Jap. p. 214 (1867).

var. *variegata* KIMURA var. nov. (Fig. 9).

Folia adulta plerumque albo-marginata et medio dilute viridi-variegata. Ceterum ut in typica. Vidi tantum ♂ stirpes.

NOM. JAP. *Hui-ri-neko-yanagi*, nom. nov.

HAB. JAPONIA. Honshû.—Prov. Rikuzen: Sendai, cult, (A. KIMURA, n. 2409 [typus var.] 24 Sept. 1936 in Herb. A. KIMURA).

23) × *Salix Turumatii* KIMURA in Sci. Rep. Tohoku Imp. Univ. 4 ser. Biol. XI. 2, p. 243 (1936) (Symbol. Iteolog. II).



Fig 9 *Salix gracilistyla* MIQUEL. var. *variegata* KIMURA. Typus

NOM. JAP. *Hitati-yanagi* TURUMATI nom. nov. in litt. ann. 1936.

24) *Salix Woodii* SEEMEN<sup>1)</sup> in ENGLER, Bot. Jahrb. XXI. Beibl. LIII. p. 53 (1896).—ENGLER, Pflanzenwelt Afrikas p. 7 (1915).—BEWS, Fl. Natal & Zululand p. 79 (1921).—DAVY in Jour. Ecology X. p. 71 (1922).

Ad descriptionem originalem adde: Gemmae steriles (in planta florente visae) in axillis adutorum foliorum ovatae apice obtusae 2–3.5 mm longae fuscae. Perula externa gemmarum ventrali latere libera et marginibus imbricatis, dorso pilis minutissimis cinereis et fulvis mixtis puberula; interna (=cataphyllum infimum) adaxialis extus puberula ut externa. Petioli eglandulosi. Folia adulta supera facie stomatifera.—Propter gemmarum structuram est subgen. *Protiteae* KIMURA subjungenda.

HAB. in Africa australi: Natal et Orange Free State. Examinaui specimen (n. 9769 ♂) a cl. J. MEDLEY WOOD lectum in Flawwhite, Albertina, O. R. C. 5–6000 ft. alt.

25) *Salix Salsaf* FORSKÅL,<sup>2)</sup> Fl. Aegypt.-Arab. p. 76 (1775).—TRAUTVETTER, Salicetum in Mém. Prés. Acad. Sc. Pétersb. Savants Étrang. III. p. 612, t. 2 (1836).—ANDERSSON in Kongl. Svesk. Vetensk. Akad. Handl. VI. 1, p. 10, t. 1, fig. 9 (1867) (Monogr. Salic.); in DE CANDOLLE, Prodr. XVI. 2, p. 196 (1868).—PAX in ENGLER & PRANTL, Natürl. Pflanzenfam. III. 1, p. 36 (1894).—ENGLER, Pflanzenwelt Afrikas p. 7 (1915).—DAVY in Jour. Ecology X. p. 71 (1922).—OPPENHEIMER, Fl. Transiordan. p. 165 (1931).

Syn. *Salix subserrata* WILLDENOW, Sp. Pl. IV. 2, p. 671 (1806).—PERSOON, Syn. Pl. II. p. 600 (1807).—POIRET, Encycl. Méth. Suppl. V. p. 58 (1817).

Gemmae anguste ovatae antice acutae, glabrae vel partim minutissime puberulae; perula gemmalis latere ventrali libera et marginibus late imbricatis. Folia adulta supera facie stomatifera. Petioli eglandulosi.—Propter gemmarum naturam primitivam in subgen. *Protiteam* KIMURA collocatur.

HAB. in Syria, Iraq, Africa (ex Angola et Rhodesia per totam Abyssiniam et Aegyptum usque in provincias littorales maris mediterranei).—

<sup>1)</sup> Hic gratias quam maximas ago cl. Dr. K. MIYABE, Professori Emerito Imperialis Hokkaido Universitatis Sapporensis, cujus benignitati hanc raritatem debeo.

<sup>2)</sup> Specimina hujus speciei ut sequentium mihi examinandi causa summa liberalitate commodaverunt Dr. E. D. MERRILL (Director Arboreti Arnoldiani), Dr. A. REHDER (Curator Herb. ejusdem Arboreti), Dr. H. A. GLEASON, Dr. G. L. WITTRICK (Curatores Herb. Hort. Bot. Noveboracensis). Omnibus his viris eorumque adjutoribus hoc loco permultas gratias agam.







*Synedrella eximata*: ad fossas prope Schubra, Kairo, (J. BORNMÜLLER n. 19041 ster. 16 V 1908 in Herb. Arb. Arnold.); S. Angola, "Tree 20-30 ft. Overhanging water on right bank of River Caculova near Njambi", (H. H. W. PEARSON n. 2011 fr. 24 V 1909 in Herb. Hort. Bot. Noveboracensis); Chehala, near Amara in Iraq, (HENRY FIELD & YUSUF LAZAR n. 33 fr. 26 Apr. 1934 in Herb. Arb. Arnold.); River Arnon, Palestina, (J. E. DINSMORE & LAVIS LARSON n. 3226 fr. "near water, tree 4 m" 20 Jun. 1910 in Herb. Arb. Arnold.).

var. *cyathipoda* ANDERSSON in DE CANDOLLE, Prodr. XVI. 2. p. 196 (1868).—ENGLER, Pflanzenwelt Afrikas p. 7 (1915).—DAVY in Jour. Ecology X. p. 71 (1922).

Syn. *Salix cyathipoda* ANDERSSON in RICHARD, Tent. Fl. Abyss. II. p. 275 (1851).

Structura gemmarum primitiva ut in typica. Folia adulta supra stomatifera. Petioli superne eglandulosi.

HAB. in Africa, e Rhodesia usque ad Abyssiniam et Eritream—Specimina visa: Kyimbila Distr., north of Lake Nyasa, (A. STOLZ n. 2130 ♂ & ♀ in Herb. Arb. Arnold.).

26) *Salix Hutchinsii* SKAN in Kew Bull. 1917 p. 235 (1917).—DAVY in Jour. Ecology X. p. 71 (1922).

Perula gemmalis ventrali latere libera et marginibus imbricatis, pertinet itaque ad subgen. *Protiteum* KIMURA.

HAB. in Africa tropica.—Vidi specimen [n 675 (T) ♂] a cl. I. R. DALE in Kenya Colony lectum et in Herb. Hort. Bot. Noveboracensis conservatum.

27) *Salix huillensis* SPEEMEN in ENGLER, Bot. Jahrb. XXIII. Beibl. LVII. p. 43 (1907).—ENGLER, Pflanzenwelt Afrikas p. 7 (1915).

Gemmae steriles in ramulo brevi (7.5 cm longo) folia adulta gerente vix; perula gemmalis ventrali latere libera et imbricata, extus pilis ferrugineis minutissimis sparse oblecta. Folia adulta supra stomatifera. Petioli eglandulosi.—Pertinet ad subgen. *Protiteum* KIMURA.

HAB. in Africa occid. et orient.-austral.—Specimina examinata: Ussupe (W. GÖRTZE n. 999 fruct. ann. 1898-1900, det. O. v. SPEEMEN, in Herb. Arb. Arnold.).

28) *Salix nepalensis* TURKHAUS, Fl. Cap. I. p. 139 (1907).—FAIRIE in Journal of the Botanical Society of London, XIII. pt. 5 p. 121

(1856) (Anmärkningar öfver de Kapska Pilarterna).—ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 13, t. 1, fig. 11 (1867) (Monogr. Salic.); in DE CANDOLLE, Prodr. XVI. 2, p. 197 (1868).—PAX in ENGLER & PRANTL, Natürl. Pflanzenfam. III. 1, p. 36 (1894).—ENGLER, Pflanzenwelt Afrikas p. 7 (1915).—DAVY in Jour. Ecology X. p. 69 (1922).

Gemmae ovatae, apice obtusae, castaneae laeves nitidiusculae glaberrimae, 2–2.4 mm longae, perulis latere ventrali liberis et imbricatis. Folia adulta supra stomatifera. Petioli eglandulosi.—In subgen. *Protiteam* KIMURA collocanda.

HAB. in Africa maxime australi capensi.—Vidi: Oranje, (K. DINTER n. 5132 ♀, 2 Jan. 1924 in Herb. Arb. Arnold.); distr. Kroonstad, (J. W. PONT n. 661 ♀ & ♂, Sept. 1929 in Herb. Arb. Arnold.).

var. *gariepina* (BURCHELL) ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 13 (1867) (Monogr. Salic.); in DE CANDOLLE, Prodr. XVI. 2, p. 197 (1868) p. p.—DAVY in Jour. Ecology X. p. 70 (1922).

Syn. *Salix Gariepina* BURCHELL, Travels in the interior of southern Africa I. p. 317, t. 6 (1822).—FRIES in Öfversigt af Kongl. Svensk. Vetensk. Akad. Förhandl. XIII. pt. 5, p. 121 (1856) (Anmärkningar öfver de Kapska Pilarterna).

Constructio gemmarum ut in typica. Folia adulta supera facie stomatifera. Petioli eglandulosi.

Specimina visa: Modderriver Station 1200 m, Capland, (O. KUNTZE ♀ 10 Feb. 1894 in Herb. Hort. Bot. Noveboracensis); sine loco spec., (DREGE ♂ & ♀ 118.9. in Herb. Hort. Bot. Noveboracensis).

29) *Salix mucronata* THUNBERG, Prodr. Pl. Cap. I. p. 6 (1794); Fl. Cap. I. p. 140 (1807).—WILLDENOW, Sp. Pl. IV. 2, p. 685 (1806).—PERSOON, Syn. Pl. II. p. 601 (1807).—SMITH in REES, Cyclop. No. 74 (1819).—SPRENGEL, Syst. Veget. I. p. 107 (1825).—FRIES, Nov. Fl. Suec. Mantissa I. p. 76 (1832); in Öfversigt af Kongl. Svensk. Vetensk. Akad. Förhandl. XIII. pt. 5, p. 120 (1856) (Anmärkningar öfver de Kapska Pilarterna).—LOUDON, Arb. & Frut. Brit. III. p. 1602 (1838).—DAVY in Jour. Ecology X. p. 70 (1922).—FLODERUS in Arkiv f. Bot. XXV. A. No. 11, p. 28 (1933).

Syn. *Salix aegyptiaca* (non L.) THUNBERG, Prodr. Pl. Cap. I. p. 6 (1794); Fl. Cap. I. p. 178 (1807).

*Salix capensis* THUNBERG var. *mucronata* (THUNBERG) ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 14 (1867) (Monogr.

Salic.); in DE CANDOLLE, Prodr. XVI. 2, p. 198 (1868).

Gemmae ovatae circiter 3 mm longae, primo cinereo-pubescentes, demum basi excepta glaberrimae, perulis ventrali latere liberis et imbricatis, marginibus ciliatis. Folia adulta supra stomatifera. Petioli eglandulosi.—Pertinet ad subgen. *Protiteam* KIMURA.

HAB. in Africa maxime australi capensi.—Examinavi specimina ex prov. austro-occid. col. Capitis Bonae Spei: Bergriver, Distr. Wellington, alt. 50 m, (R. MARLOTH n. 4283 ♂, tree 10 m high, March 1906); Bonnie vale, Distr. Swellendam, bank of Breede river, alt. 150 m, (R. MARLOTH n. 11843 ♀ & ♂, Oct. 1st 1923); Ceres, banks of river, alt. 500 m, (R. MARLOTH n. 6392 ster. March 1925).—Omnia in Herb. Arb. Arnoldiani asservata.

30) *Salix* subgen. *Protitea* sect. *Acmophyllae* ANDERSSON suppl. KIMURA.

Syn. *Salix* I. *Amerina* ANDERSSON in Svensk. Vetensk. Akad. Handl. ser. 3, 1850 p. 470 (1851) (Ost-Indiens hittills kända Pilarter) quoad *S. dealbatam*.

*Salix* tribus A. *Salices Pleiandrae* a. *Tropicae* stirps II. *Salices orientales* v. *S. acmophyllae* ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 7 (1867) (Monogr. Salic.) quoad *S. acmophyllum* & *S. dealbatam*.

*Salix* A. *Salices Pleiandrae* 1° *Tropicae vel subtropicae* § 2. *Orientales vel acmophyllae* ANDERSSON in DE CANDOLLE, Prodr. XVI. 2, p. 195 (1868) quoad *S. acmophyllum* & *S. dealbatam*.

*Salix* sect. I. *Pleiandrae* HOOKER, Fl. Brit. Ind. V. p. 626 (1888) quoad *S. acmophyllum*.—BRANDIS, Ind. Trees p. 636 (1906) quoad *S. acmophyllum*.

*Salix* sect. 4 *Acmophyllae* ANDERSSON ex SCHNEIDER in SARGENT, Pl. Wilson. III. p. 105 (1916).

Descriptioni a cl. SCHNEIDER datae adde: Gemmae 1-perulatae, perulis ventrali latere liberis et imbricatis. Folia adulta supera facie stomatifera. Petioli eglandulosi.—In subgen. *Protiteam* KIMURA collocatur.—Examinavi speciem typicam; vide infra.

31) *Salix acmophylla* BOISSIER, Diagn. Pl. Orient. ser. I. fasc. VII. p. 98 (1846); Fl. Orient. IV. p. 1183 (1879).—ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 7, t. 1, fig. 6 (1867) (Monogr. Salic.); in DE CANDOLLE, Prodr. XVI. 2, p. 195 (1868).—HOOKER, Fl. Brit. Ind. V. p. 628 (1888) p. p.—BRANDIS, Ind. Trees p. 636 (1906).—HANDEL-MAZZETTI in Annal. k. k. Hofm. Wien XXVI. p. 129 (1912).—NABELEK,

Iter Turc.-Pers. IV. in Publ. Fac. d. Scienc. Univ. Masaryk CV. p. 24 (1929).—GÖRZ in FEDDE, Rep. Sp. Nov. Reg. Veg. XXVIII. p. 79 (1930) (K. KRAUSE, Beiträge zur Flora Kleinasiens. V.).

Syn. *Salix Persica* BOISSIER, Diagn. Pl. Orient. ser. I. fasc. VII. p. 99 (1846); Fl. Orient. IV. p. 1183 (1879).—ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 9, t. 1, fig. 7 (1867) (Monogr. Salic.); in DE CANDOLLE, Prodr. XVI. 2, p. 195 (1868).—SCHISCHKIN in Ber. d. Tomsk. Staats-Univ. LXXX. p. 436 (1929).

*Perula gemmalis* ventrali latere libera et imbricata. Folia supra facie stomatifera. Petioli eglandulosi.—Subgen. *Protiteae* KIMURA submittenda.

HAB. in Persia, Mesopotamia, Syria, India.—Specimina visa: Mesopotamia. Biredjik: Haschnadi, ad Euphratem, (P. SINTENIS n. 491 fr. 24 IV 1888, det. Dr. O. STAPP).—Persia bor., alpes Elbrus, supra Ferasad, 1700 m, (J. BORNMÜLLER n. 8260 fr. 28 V 1902).—Persia austro-orient. prov. Kerman: Kerman, ad pagum Baghin, c. 2000 m. s. m. (J. BORNMÜLLER leg. & det. n. 4550, 18 IV 1892 sub nom. *S. persicae* BOISSIER).—Omnia in Herb. Arboreti Arnoldiani.

32) *Salix* (subgen. *Protitea*) *Dunnii* SCHNEIDER in SARGENT, Pl. Wilson. III. p. 97 (1916).—SHUN-CHING LEE, Forest Bot. China p. 181 (1935).—KIMURA in Sci. Rep. Tōhoku Imp. Univ. 4 ser. Biol. XI. 2, p. 249 (1936) (Symb. Iteolog. II.).

Folia adulta supra non stomatifera.—Vidi typum in Herb. Arboreti Arnoldiani!

HAB. China. Fokien: sine loco spec.; "Province of Fokien, China. Collected on Mr. DUNN's expedition to Central Fokien, April to June 1905. Hongkong Herbarium, No. 3504."—Numerus manu scriptus hujus speciminis 3504 nec 3509 esse videtur; confer SCHNEIDER l. c. p. 98.

#### CORRIGENDUM

Symbolae Iteologicae II in Sci. Rep. Tōhoku Imp. Univ. 4 ser. Biol. XI. no. 2.

Pag. 251, lin. suprema, loco emend., lege suppl.

## EXPLICATIO TABULARUM.

## TAB. III.

*Salix cremnophila* KIMURA. Typus.

## TAB. IV.

*Salix cremnophila* KIMURA.  
Ramuli amentiferi.

## TAB. V.

*Salix Isikawae* KIMURA. Typus.  
Ramuli amentiferi.

## TAB. VI.

*Salix Isikawae* KIMURA.  
Ramuli cum foliis adultis.

## TAB. VII.

*Salix sirakawensis* KIMURA. Typus.  
Ramuli amentiferi.

## TAB. VIII.

*Salix sirakawensis* KIMURA.  
Ramuli cum foliis adultis.

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HERB. ARIKA KIMURA

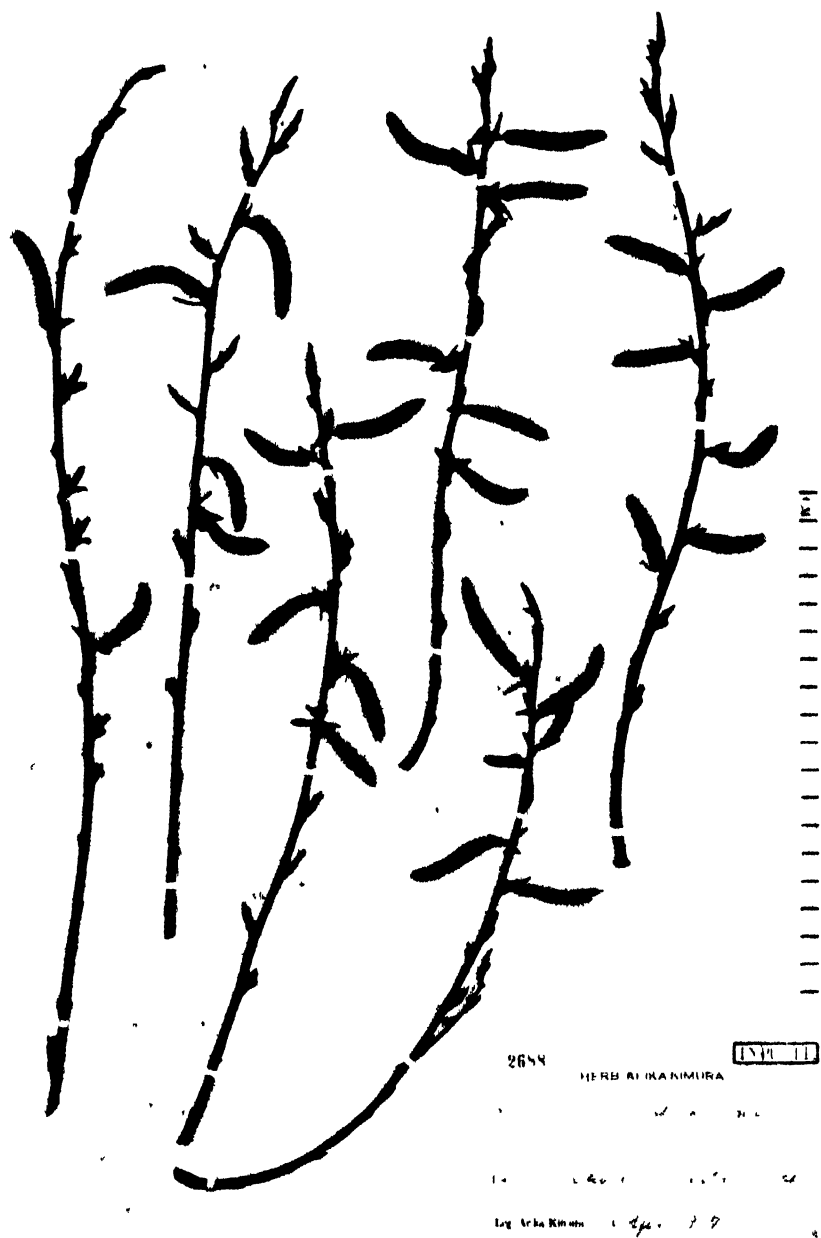
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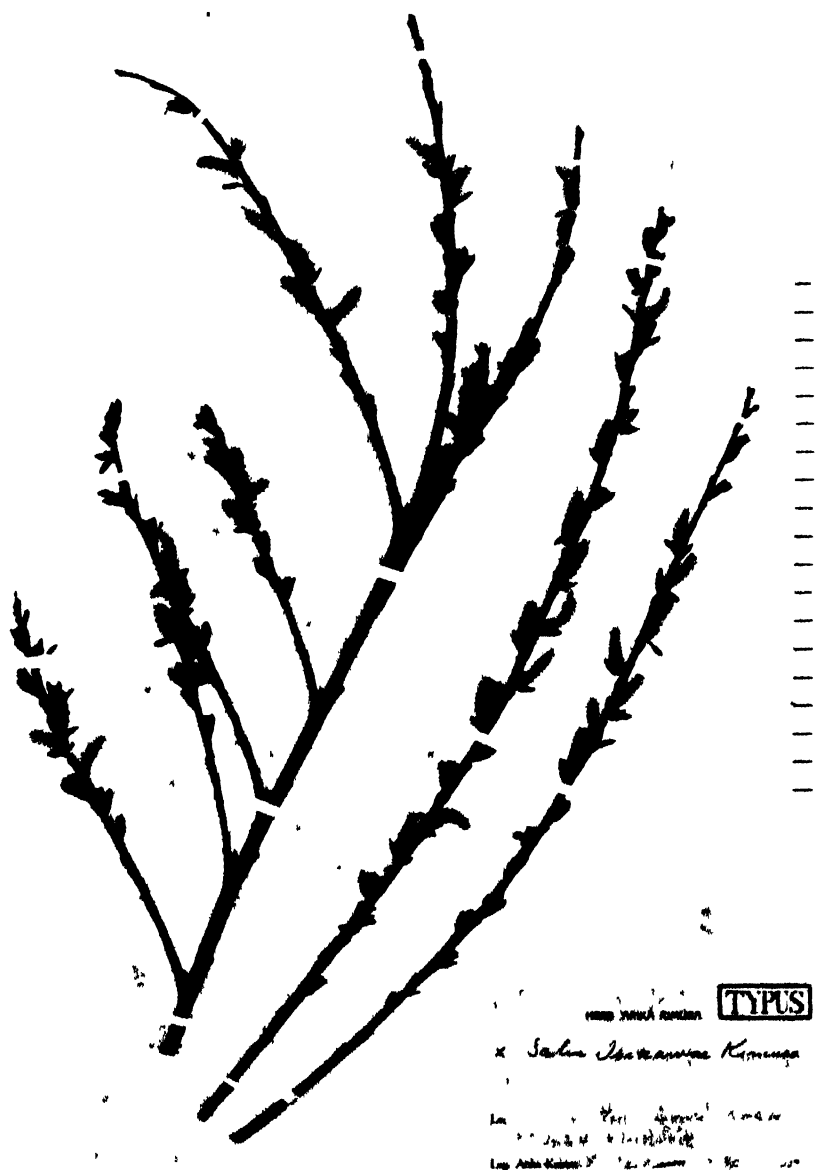
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 Leg. Arika Kimura 1910. 12. 25.











A. KIMURA: Symbolae Iteologicae III.





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A. KIMURA: Symbolae Iteologicae III







A. KIMURA: Symbolae Iteologicae III.



# **SOME NOTES ON THE GASTRULATION AND THE FORMATION OF GERMINAL LAYERS IN THE SNAKE, *ELAPHE QUADRIVIRGATA QUADRIVIRGATA* (BOIE)**

By

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(With Plates IX-X and seventeen figures in text)

(Received June 16, 1937)

The process of gastrulation and the formation of germinal layers in some reptiles have been studied with special interest, and these processes, especially in the case of *Lacerta* and of *Chelonia*, have already been ascertained and determined by many investigators.

The modus of gastrulation and the establishment of germinal layers in snakes have previously been observed, so far as the present writer is aware, by KUPFFER (1882), HOFFMANN (1887-1890), WILL (1899), GERHARDT and O. HERTWIG (1901), and by BALLOWITZ (1901-1902). The specimens, on which these investigators worked, were *Vipera (Peliás) berus* L., *Coluber aesculapii* STURM and *Tropidonotus natrix* BOIE. In particular, the last one was taken as a favourable specimen to determine the processes above-mentioned, which were dealt with various conclusions. Since their day no further investigations in regard to the early developmental stages of snakes have been published.

As hitherto no investigations have been carried out with regard to Japanese snakes, the present writer has ventured to attempt a serial investigation of their development.

In the present paper, which deals with the Japanese striped snake ('shima-hebi'), the present writer wishes to give the results of his own direct observation of the formation of the blastopore and of the germinal layers and of the process of the completion of the 'blastoporic passage,' or Kupfferian canal, referring to those obtained by the earlier investigators.

The present writer has here to record his cordial thanks to Prof. Dr. E. NOMURA and also to Messrs. K. OKADA and I. HAMAI for their helpful assistance and useful advice during the progress of the present research.

## MATERIAL AND METHOD

The Japanese striped snake, *Elaphe quadrivirgata quadrivirgata* (BOIE), was used as the material. The species is widely distributed all over the mainland of Japan.

Pregnant females of this species were collected in the suburbs of Anjô, a town near the city of Nagoya. In this locality, the breeding season of this species extends usually from the middle of June to that of July, copulation taking place in the latter part of May. All the females, from which the embryos were obtained, were captured freshly from the wild habitat, and in no case had any of them been kept in a vivarium. The objection to using cultured specimens is due to the fact that, under the unnatural conditions in the vivarium, the embryos would die and decompose in the maternal oviducts. Therefore, the animal was chloroformed and killed immediately after being captured, and the abdomen was then opened in order to obtain the eggs.

The eggs, which are arranged moniliformly end to end, were first removed by dissecting the oviducts of the pregnant female, and then, after careful removal of the tough shell envelope, the blastoderm was cut round with a pair of fine scissors, the large subgerminal fluid cavity immediately below being deeply pierced through. The excessive adhesion of yolk matter, which tends to clog the sectioning, was cautiously removed as far as possible from the blastoderm with a spatula.

After finishing the above method of treatment, the blastoderm spread on a spatula was, at the outset, put in a saturated aqueous solution of picric acid for a quarter an hour. It was then hardened for half an hour with a modification of Rabl's picro-sublimate, i. e. a mixture of equal parts of a saturated aqueous solution of picric acid and of corrosive sublimate. After fixation, the material was transferred to a 70% alcohol, containing iodine, for a day, and then preserved in an 85% alcohol.

For staining, Delafield's haematoxylin and eosin were used for some of the material, but, for the greater part of it the total staining method with Grenacher's borax-carmines was preferred. In order to ascertain the superficial relief in the region of the blastopore, a material treated with the latter stain was observed under a microscope through reflected light.

Finally, the material was sectioned, 10  $\mu$  in thickness, by the paraffin method. The transverse and longitudinal serial sections were thus prepared for the purpose of investigation.

## SPECIFICATION OF STADIA

The material at the disposal of the present writer was lacking in the stages, which might have demonstrated the earlier developmental conditions before the formation of the primitive plate.

In this paper, the stages of the subsequent development are described, i. e. from the first indication of the blastopore up to the completion of the blastoporic passage, or Kupfferian canal.

As the process of gastrulation is proceeding, the form of the blastopore and the deepening of the invagination cavity are gradually changing in appearance, giving a characteristic feature to each stage. The present writer proposes, therefore, to specify the stages of the whole process of invagination in five stadia describing the developmental conditions for the sake of convenience.

The characteristics of each stadium are as follows, namely ---

*Stadium I.* There is present, on the posterior margin of the embryonic shield, a definite primitive plate, on which the superficial ectodermal epithelium has just begun to invaginate, and, consequently, prosperous inward proliferation is taking place. The parablasic cells are forming a distinct layer, there being a space between them and the pre-existing superficial layer. The anterior and posterior lips of the blastopore are almost the same in height, or the latter somewhat exceeds the former. The state of the shallowly invaginated groove presents a typical archistome<sup>1)</sup>.

*Stadium II.* The shallow groove above-mentioned deepens, and its bottom turns in the antero-ventral direction. In this second stadium, as the invagination cavity is often much expanded, the blastoporic opening appears somewhat narrow. The underlying primary endoderm, which is now in the course of establishment, represents a fairly complete condition. The anterior lip of the blastopore rather exceeds the posterior lip in height. The invagination cavity of this stadium shows a transitional condition from an archistome to a prostome<sup>2)</sup>.

*Stadium III.* The deepening of the invagination cavity is directed anteriorly and attains a nearly full-grown condition, in which the cavity has an anterior expanded part and a posterior dorso-ventrally flattened outlet. The floor, with the exception of the anterior and posterior ends of the cavity, represents a sheet of cells, by which the invagination cavity and the subgerminal fluid cavity are separated. The roof, on the other hand, shows an active division of cells. The peristomial mesoderm begins

1) and 2) The technical terms follow BALLOWITZ.

to proliferate in the posterior margin of the primitive plate. The anterior lip barely overlaps the posterior one, indicating a typical prostome.

*Stadium IV.* This corresponds to the stages of the 'breaking through'<sup>1</sup> of the invagination cavity into the underlying subgerminal fluid cavity by the disappearance of the sheet of cells, which separates both cavities. The chorda primordium is differentiated from the dorsal wall of the invagination cavity, being by degrees established anteriorly. The primitive plate becomes rather flat, decreasing in thickness and slanting posteriorly. In the turned-in region of the anterior lip is often found a remarkable intumescence of cells, which is a temporary structure and soon disappears.

*Stadium V.* In this stadium, the blastoporic passage, or Kupfferian canal, begins to shorten, and its anterior opening is in the course of obliteration. The formation of the chorda anlage, particularly of the gastral mesoderm, and a characteristic inlet of the Kupfferian canal, occurring in the region of the original posterior lip, give a peculiarity to this stadium, which represents a transitional structure from a prostome to a metastome<sup>2</sup>.

## OBSERVATIONS

### STADIUM I

The earliest specimen at the disposal of the present writer had a distinct embryonic shield, measuring approximately 2 mm. in median longitudinal diameter. A definite primitive plate was already present along the hindermost periphery of the shield, and is represented as a simple localized thickening of the superficial ectoderm.

The superficial ectoderm, covering a larger area of the embryonic shield, is already a layer of two- or three-cell thickness. The cells, constituting this layer, are arranged in a columnar manner. The columnar arrangement of cells is most distinct in the region immediately in front of the primitive plate, and becomes more irregular and indistinct towards the anterior and peripheral limits of the embryonic shield. The surface of the ectoderm is flat, and the underface of it, therefore, shows an unevenness according to the irregularity in the arrangement of the cells. The process of vertical elongation of columnar cells originates first at the anterior boundary of the primitive plate, and extends gradually to the anterior and lateral portions of the shield. In the larger part of the

<sup>1</sup> This term is used, following MITSUKURI, in the place meaning the special perforation in the floor of the invagination cavity.

<sup>2</sup> The technical term follows BALLOWITZ.

embryonic shield, with the exception of the anterior zone of the primitive plate, the position of the nuclei of cells, which form the epithelium, are irregular, and appear as if they were only scattered in the superficial ectoderm, accompanying the irregularity in arrangement of the cells. The

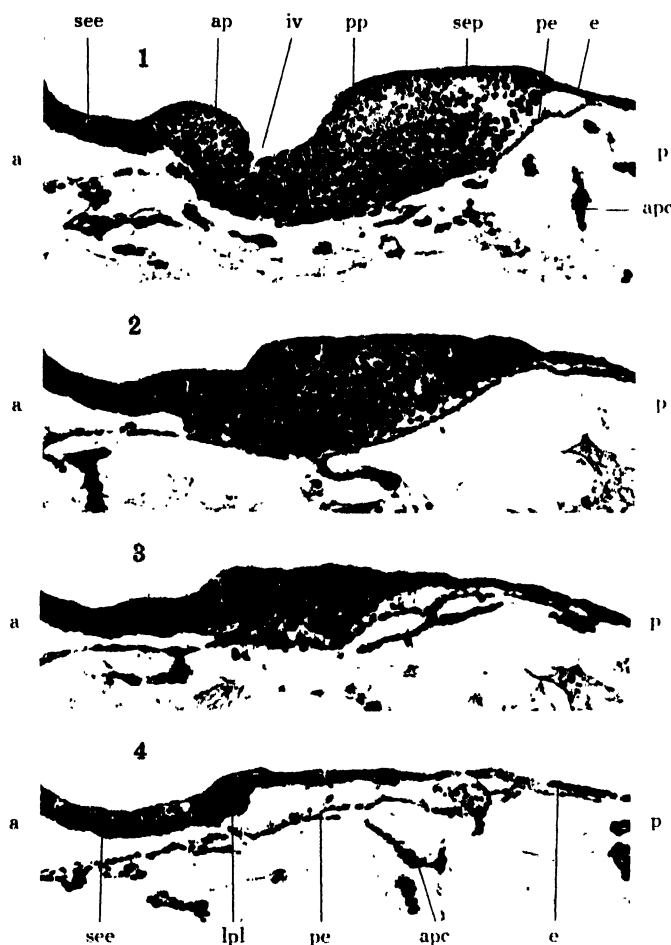


Fig. 1. Photomicrograph of longitudinal sections of specimen early in Stadium I, in order of 1-4 from median to right, showing relation between invagination cavity and adjacent tissues, especially between heights of anterior and posterior blastoporic lips.  $\times 80$ . *a* anterior direction, *ap* anterior blastoporic lip, *apc* aggregation of parablastic cells, *e* ectoderm of annular zone, *iv* invagination cavity, *lpl* lateral margin of primitive plate, *p* posterior direction, *pe* primary endoderm, *pp* posterior blastoporic lip, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.



thickness of the ectoderm varies according to the part of the embryonic shield which it covers. It is thickest at the immediate front of the primitive plate, which shows an accurate columnar structure, but, then, tends to decrease anteriorly and laterally. In the central part of the embryonic shield, the epithelium measures about  $27\ \mu$  in thickness. Except in the region posterior to the primitive plate, the ectoderm by degrees decreases in thickness towards the annular zone of the embryonic shield, until it passes continuously into a layer of a one-cell thickness, which consists of somewhat cubical cells, and, finally, in the further peripheral portion, it alters into a layer of dorso-ventrally flattened cells (I, III, VI and VII in Pl. IX). Thus, the differentiation of the superficial ectoderm into the embryonic shield and adjacent region is indistinct, but, on the other hand, in the region immediately posterior to the primitive plate, the superficial layer combines directly with the unicellular layer, consisting of cubical cells (Figs. 1, and 2, and III in Pl. IX).

Meanwhile, a shallow transverse groove (Fig. 1<sub>1-4</sub>) appears just on the anterior margin of the primitive plate, gradually diminishing in depth towards both lateral ends. This groove is the first indication of the invagination, and corresponds to the 'Sichelrinne' of WILL (1899) and to the 'Archistom' of BALLOWITZ (1901). The posterior lip of this invagination exceeds the anterior one in height (Figs. 1, 2 and 3). On the anterior wall, on the bottom, and on a part of the posterior wall of the invagination cavity, may be observed a columnar cell-arrangement, such as is seen in the superficial ectoderm anterior to the primitive plate, even though it is indistinct, and becomes more indistinct towards the upper part of the posterior wall, finally disappearing at the top of the posterior lip. That is to say, the cells in this region represent active cell-division, and really mitotic figures are frequently observed. On the surface posterior to the posterior lip, the columnar arrangement of cells is not found, this portion consisting only of somewhat cubical cells, as seen in the superficial layer of the posterior continuation of the primitive plate.

In the region of the primitive plate, the distinction between the superficial and subjacent ectodermal cells is not, more or less, clear. Morphologically, the superficial cells resemble the subjacent cells composing the substance of the primitive plate. The latter cells are, however, scattered rather loosely, leaving numerous intercellular spaces between them. The former cells, on the contrary, represent a coherent condition. Thus, it is scarcely possible to distinguish them from each other merely from the difference in the arrangement of the cells (Figs. 1, and 2). The subjacent

cell-mass or the 'Blastemgewebe der Stomaplatte' of BALLOWITZ is presumed to be derived mainly from the region extending from the posterior lip to the posterior wall of the invagination cavity.

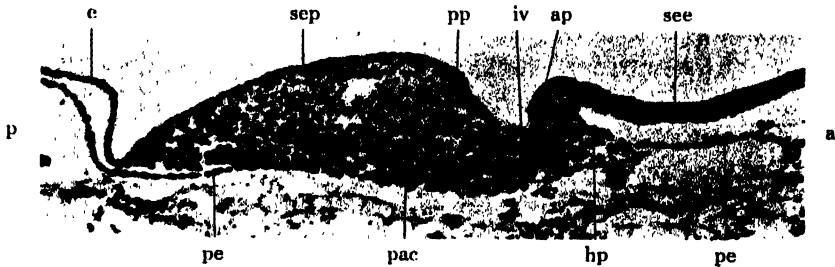


Fig. 2. Photomicrograph of median longitudinal section of specimen in Stadium I, slightly later than that shown in Fig. 1, showing relation between head process and invagination cavity. Posterior blastoporic lip surpasses anterior one slightly in height.  $\times 80$ . *a* anterior, *ap* anterior lip, *apc* aggregation of parablasic cells, *e* ectoderm of annular zone, *hp* head process, *iv* invagination cavity, *p* posterior, *pac* portion of active cell-division, *pe* primary endoderm, *pp* posterior lip, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.

Hitherto, the walls of the invagination cavity have been variously termed, viz. 'Urdarmblatt' by GERHARDT and O. HERTWIG, 'primäre Entoderm' or 'Ur-Entoderm' by KUPFFER, 'palingenetische Entoderm' by WENCKEBACH, etc. But, in this paper, the present writer wishes to use consistently the term 'secondary endoderm' in its ontogenetic meaning.

In the course of development, from the anterior extremity of the primitive plate, a group of cells, which are derived from the anterior wall of the invagination cavity, are encroaching forwards, forming a small

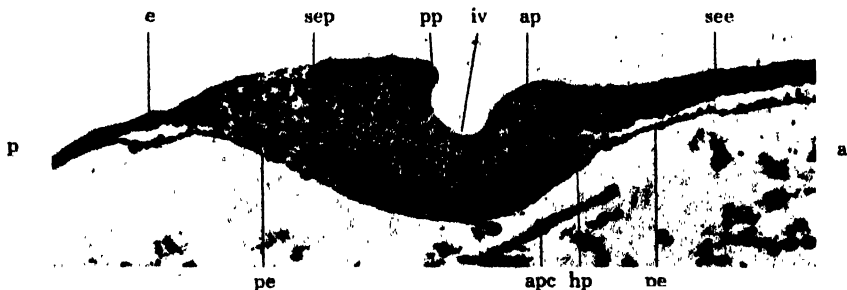


Fig. 3. Photomicrograph of median longitudinal section of specimen in Stadium I, slightly later than that in Fig. 2, showing considerably expanded invagination cavity directed somewhat posteriorly. Posterior blastoporic lip surpasses anterior lip apparently in height.  $\times 80$ . Abbreviations as before.

process between the superficial ectoderm and the underlying cell-layer, which extends to a definite distance from the ectoderm in the region of the embryonic shield. This small process projecting anteriorly is the forerunner of the head process (Figs. 2 and 3). The underlying layer spreads horizontally all over the blastoderm and consists of thin, flat, unicellular strips, which are arranged transversely, crevices remaining between them. Each strip consists, in turn, of loosely arranged, spindle-shaped cells. Thus, this layer, in this stage, is perforated by numerous passages that appear, in sections, as breaks in a sheet.

In the early specimen (Fig. 2), the subgerminal fluid cavity spacious in extent had been already completed under the whole area of the blastoderm. The ventral surface of the primitive plate is still flat, but it becomes gradually protruded towards the cavity, owing to the increase in bulk of the cell-mass of the primitive plate (Figs. 3 and 4). The downward thickening of the cell-mass occurs not only in the median zone of the invagination cavity, but also in the lateral parts, though it is slight (Figs. 1<sub>2</sub> and 3 and 4<sub>2-4</sub>), i. e. the activity of cell-division is most vigorous in the median part and, gradually, weakens laterally.

As described above, underneath the blastoderm, extends the layer of spindle-shaped cells, which are still, in this stage, arranged very incompletely, as indeed they are in an earlier stage. These cells, in a later stage, are to form a definite unicellular layer, which has been, hitherto, called by various terms by several investigators, as may be seen later in the section of 'Remarks,' but in this paper the present writer wishes to use uniformly the term 'primary endoderm,' for convenience of description. The shape and size of cells, constituting the strip, vary very much, viz. they are elliptical or spindle-shaped, larger or smaller, etc. The anlage of the primary endoderm extends here, cohering with the underface of the primitive plate and of the superficial ectoderm at the annular zone of the blastoderm, with the exception of the region of the embryonic shield (III in Pl. IX). On the peripheral zone, the cells of the primary endoderm adjoin other cells, being actively proliferated from the germ wall. In this stage, it is noticeable that some primary endodermal cells are being attached to the superficial ectodermal cells, as if the former were delaminating from the latter. Such attachments are observed, here and there, in the periphery of the embryonic shield, particularly in the region posterior to the primitive plate (IV and V in Pl. IX). From these facts, it appears to the present writer that the cells, forming the primary endodermal layer, originate chiefly from the parablastic cells, which will be described later,

and are added to, even though these are only a few, by those derived from the germ wall and, probably, from the ectoderm of the annular zone. In



Fig. 4. Photomicrograph of longitudinal sections of specimen in Stadium I, of almost the same age as in Fig. 3, in order of 1-4 from median to right, showing remarkably protruded underface of primitive plate and poorly developed head process. Invagination cavity is directed posteriorly, as seen in Fig. 3.  $\times 80$ . *sg* transverse shallow groove between primitive plate and annular zone, *lpl* lateral margin of primitive plate. Other abbreviations as in Fig. 2.

the region of the primitive plate, immediately ventral to it, the primary endoderm appears to combine with the cell-mass of the plate, but, to be

precise, the distinction between them is recognizable, i. e. the cells, constituting the plate, are generally spherical in shape and loosely associated, but those of the primary endoderm are, on the other hand, spindle-shaped and the cytoplasm and nuclei are deeply stained. During the present stadium, the primary endodermal cells do not yet form a complete layer in the region of the primitive plate as also in the other region, consequently, the ventral surface of the plate faces directly the subgerminal fluid cavity, though only partially. This observation elucidates the fact that the coherence of both elements, the primary endodermal cells and the cell-mass of the primitive plate, is merely caused by the downward growth of tissue of the plate itself, owing to the increase in the number of cells, and no migration of both elements was observed anywhere.

Underneath the blastoderm, lies the spacious subgerminal fluid cavity, in which are scattered a large number of free cells of several sizes. Moreover, in this cavity, near its floor, are found masses of yolk or yolk spheres, some of these being embedded in the superficial zone of yolk, their contour being clearly outlined (Fig. 1). All the cells, which are scattered freely in the subgerminal fluid cavity, are obviously the descendants of yolk cells. This fact is clear when the periblast of yolk is accurately observed. According to these facts, the freely scattered cells appear to belong, without exception, to the endoderm. Merocytes, or yolk-syncytia, are observed on the surface of the yolk, facing the cavity, particularly in the region of the germ wall. On the whole surface of the yolk, nuclei of the syncytia are in various degrees of formation of parablasic cells, being embedded in the yolk substance (I in Pl. IX). Parablasic cells, have been just completed, are proliferated into the subgerminal fluid cavity, so that comparatively large cells, containing a large quantity of yolk matter, come to be found scattered in the cavity. These parablasic cells may be distinguished as being of two kinds, viz. one, the spherical and extraordinarily large cells, containing a considerable number of yolk globules, a small amount of cytoplasm and a small nucleus, which is deeply stainable, and the other, the elliptical cells, smaller than the spherical ones, having a small amount of yolk element and a granular cytoplasm round the nucleus (I in Pl. IX). Vacuoles are often present in these cells. The fact, that the larger yolk-laden cells are proliferated later than the smaller, is suggested by the evidence, viz. that the former cells are generally situated in the lower part of the cavity, and the cells, now forming in the yolk, belong to this type. Some of the smaller cells, which are found in the comparatively upper part of the cavity, form irregular aggregations,

scattered here and there. Some remain isolated. Other ones attach themselves to the primary endoderm, which is a flat sheet consisting of strips. In reality, some smaller parablasic cells are forming irregular, thread-like aggregations, which mainly hang from the margins of each strip, so that the lower surface of the primary endoderm is uneven. From the facts, that the primary endoderm is not a simply unicellular but an irregularly unicellular epithelium, that the connection of the cell-aggregations to the strips is firm, and that both cells are allied to each other morphologically, it may be well assumed that the parablasic cells participate in the formation of the primary endoderm from fairly early stages (II and VII in Pl. IX). The connection between the primary endoderm and the smaller parablasic cells is found not only in the region of the embryonic shield, but also in the annular zone of the blastoderm (III, IV and VI in Pl. IX). The aggregation of parablasic cells has already been observed by many previous investigators.

In the spacious subgerminal cavity, containing a large number of parablasic cells, there are also found greatly irregular, fine fibrillar networks, connecting the cells with each other (I in Pl. IX). These are the artifacts which were made in the course of fixation.

With the progress of development, the median longitudinal diameter of the embryonic shield somewhat elongates, and the superficial ectodermal layer becomes better defined, i.e. it increases generally in thickness, measuring approximately  $40\mu$  in the central region of the shield, and the differentiation between this shield and the adjacent regions becomes distinct, owing to the reservation of the original thickness in the annular zone. Moreover, the shield is lifted more or less above the annular part (III and VI in Pl. IX), and the formation of the underlying, primary endoderm advances more than it does in the earlier stages. In this stage, the primary endoderm is forming a definite layer maintaining a constant distance from the superficial ectoderm.

Columnar cells, constituting the ectoderm of the embryonic shield, form a distinct epithelium, and the nuclei occupy a basal position. This appearance is most remarkable in the region anterior to the anterior blastopore lip, and becomes more indistinct anteriorly and antero-laterally, with the extension of the thickening of the shield ectoderm.

The invagination cavity becomes deeper than that at the earlier stage, and is shown as a shallow groove with a roundly expanded end in a median longitudinal section. The bottom of the invagination cavity is directed somewhat posteriorly, but this is a temporary movement, as may

be clear when the invagination cavity of this stage is compared with that at the other stages (Figs. 2, 3, 4, and 5). The walls of the invagination cavity, with the exception of the region of the posterior lip and the zone slightly inside it, consist of columnar cells as the continuation of those of the embryonic shield.

The anterior and posterior lips are approximately the same in height, showing a form of typical archistome (Fig. 1.).

The surface of the primitive plate, posterior to the posterior lip, is flattened as in the previous stage, and, at the transitional part to the annular zone of the blastoderm, a shallow transverse groove makes its appearance. This groove is most remarkable in the median, but shallows gradually to both lateral parts (Fig. 4<sub>1-1</sub>). The groove in question has always been found in all specimens in the present stadium, which were fixed satisfactorily. Notwithstanding this, BALLOWITZ was able to find it in a few specimens belonging to corresponding stages, and stated it to be a special case.

The undifferentiated cell-mass in the primitive plate is compact and shows a conspicuous activity of cell-division. The ventral surface of this mass protrudes more prominently towards the subgerminal fluid cavity than that of the earlier stage, and this condition is most remarkable in later stages (Fig. 4<sub>1</sub>).

The cell-aggregations in the subgerminal fluid cavity grow further, and are still connected, here and there, with the underface of the endodermal layer.

It is worthy of note, particularly, in the comparatively earlier stages, that the degree of formation of the invagination cavity and that of the primitive plate are not quite correlated. In reality, the present writer has often met with specimens, which have a feebly developed primitive plate and a strongly developed invagination cavity, and vice versa. On the other hand, the correlation between the degree of formation of the primitive plate and of the rudiment of the head process is not similarly recognizable (Figs. 3 and 4<sub>1</sub>).

From the above statements, it may be said that there is great variability as to these conditions in the material as regards Stadium I.

## STADIUM II

In the stage a little later, the somewhat posteriorly directed invagination cavity deepens more, and the anterior limit of the bottom begins to

bulge in an anterior direction. Consequently, the presence of the head process becomes more or less distinct (Fig. 5). The walls of the invagination cavity, with the exception of the upper half of the posterior wall, are clearly defined in the columnar arrangement of the cells. The anterior lip is gradually raised dorsally to this stage, and, finally, more or less, exceeds the posterior lip in height. This movement of the heightening of the anterior lip is mainly caused by a throwing up due to the forward deepening of the invagination cavity and to the forward insinuation of the cells at the anterior extremity of the primitive plate. The formation of the head process is most remarkable along the median line of the embryonic shield, constituting the forerunner of the chorda dorsalis.

The cell-mass of the primitive plate extends somewhat laterally and posteriorly, so that the plate itself becomes longer and flatter than that in earlier stages (Figs. 4, and 5). The downward growth of the plate,

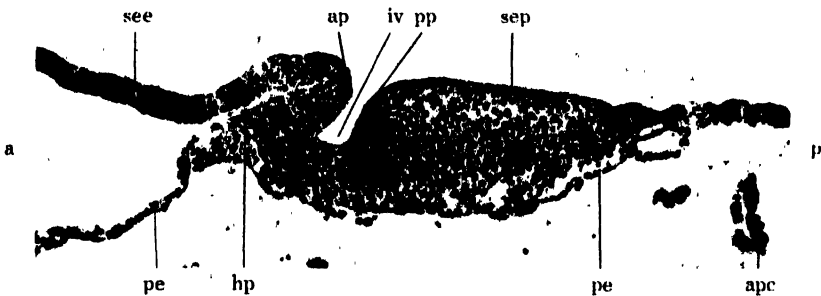


Fig. 5. Photomicrograph of median longitudinal section of specimen early in Stadium II, showing anteriorly directed short invagination cavity and flat primitive plate. Head process develops normally. Anterior blastoporic lip surpasses posterior lip slightly in height.  $\times 80$ . Abbreviations as in Fig. 2.

already mentioned, ceases, and the next stage is characterized by the formation of the peristomial mesoderm. The cells of the primitive plate show similar conditions to those in the earlier stages, but, in the undermost part, several intercellular spaces are observed. In the central, most compact part of the plate, mitotic figures are frequently observable. The upper surface of the primitive plate is flat in general, and slants posteriorly until its posterior border is bounded by a shallow transverse furrow (IV in Pl. IX). The distinctness between the cells forming the plate and those of the underlying endoderm becomes less clear than in the earlier stages.

In the case of some specimens in this stage, it is noticeable that the



endodermal layer shows a greatly irregular unevenness in the posterior half of the primitive plate and a honey-combed structure in sections, owing to an active addition of parablastic cells. In the region further posterior to the plate, the adherence of the parablastic cells in the form of aggregations, is particularly remarkable (IV in Pl. IX). At the boundary between the primitive plate and the annular zone, the relation between the endoderm and the superficial ectoderm is more intimate, and the protoplasmic processes of the former often reach the latter (IV and V in Pl. IX). This relation is most evident in the more lateral sections.

Some of the parablastic cells forming aggregations and the isolated ones in the subgerminal cavity have the appearance of decomposing.

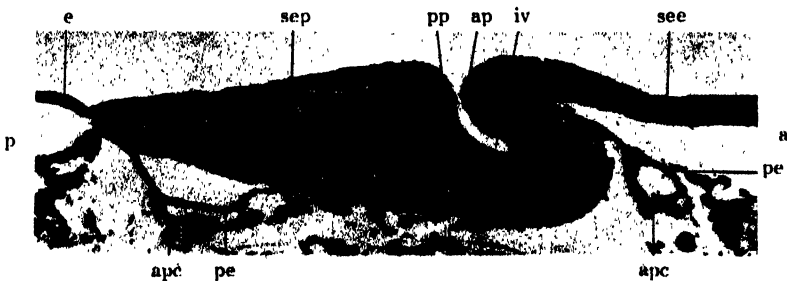


Fig. 6. Photomicrograph of median longitudinal section of specimen in Stadium II, slightly later than that shown in Fig. 5, showing noticeably narrow invagination cavity. Primary endoderm creeps dorsally along anterior surface of turned-in wall of invagination cavity.  $\times 80$ . Abbreviations as in Fig. 2.

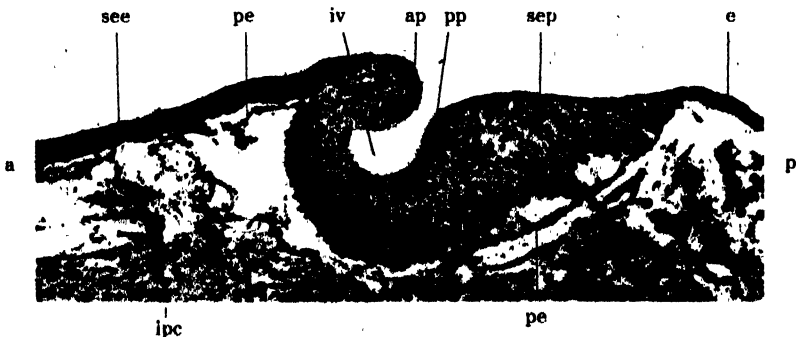


Fig. 7. Photomicrograph of median longitudinal section of specimen in Stadium II, of same age as that in Fig. 6, showing extraordinarily expanded invagination cavity. Attachment of primary endoderm to anterior surface of turned-in wall of invagination cavity is in the same condition as in Fig. 6.  $\times 80$ . *ipc* isolated parablastic cell. Other abbreviations as in Fig. 2.

Even if the development proceeds, the extension of the embryonic shield and the structure and thickness of the superficial ectodermal layer show no notable changes, when compared with those in earlier stages. So it is not necessary to describe these features here. Subsequent morphological change in the blastoderm is principally restricted to that in the invagination cavity and in the head process.

The invagination of the archistome progresses further, i. e. the anteriorly directed invagination at the bottom advances more anteriorly. In this case, it is noticeable that there are great variations in the shape of its distal part. In one specimen (Ser. 20), the bottom of the invagination cavity expanded much in a somewhat dorsal direction, and showed a round cavity accurately distinguished from the narrow blastoporic region (Fig. 7). In another (Ser. 24), on the contrary, the invagination cavity was very narrow, both the distal part and the outlet having the same calibre (Fig. 6). A transitional case (Ser. 28) was also found (Fig. 8). In a

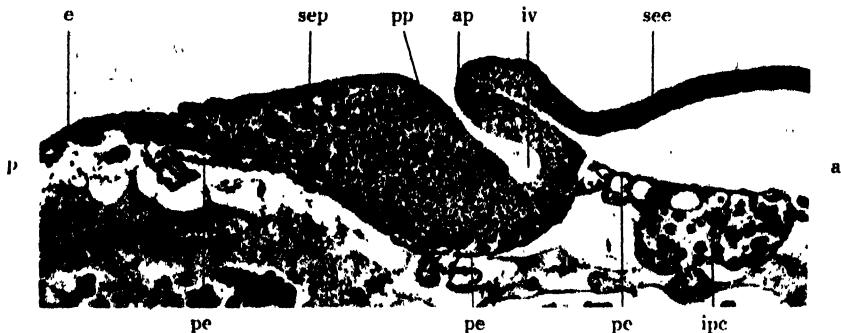


Fig. 8. Photomicrograph of median longitudinal section of specimen in Stadium II, slightly later than that shown in Fig. 7, showing a most remarkable coherence of parablastic cells to primary endoderm in portion anterior to primitive plate.  $\times 80$ . *ipc* isolated parablastic cell. Other abbreviations as in Fig. 2.

normal case in Stadium II, the blastoporic opening becomes somewhat narrower than that in the earlier stage, and this reduction in calibre of the opening is caused mainly by the backward growth of the anterior lip directed dorso-caudally.

A median small, but remarkable, cellular process was, in a few cases, observed on the posterior lip, directed anteriorly towards the blastoporic opening (Fig. 9, and IX<sub>s</sub> and D in Pl. X). No explanation of its significance has as yet been found.

In the stages succeeding the above, the region which has been previous-

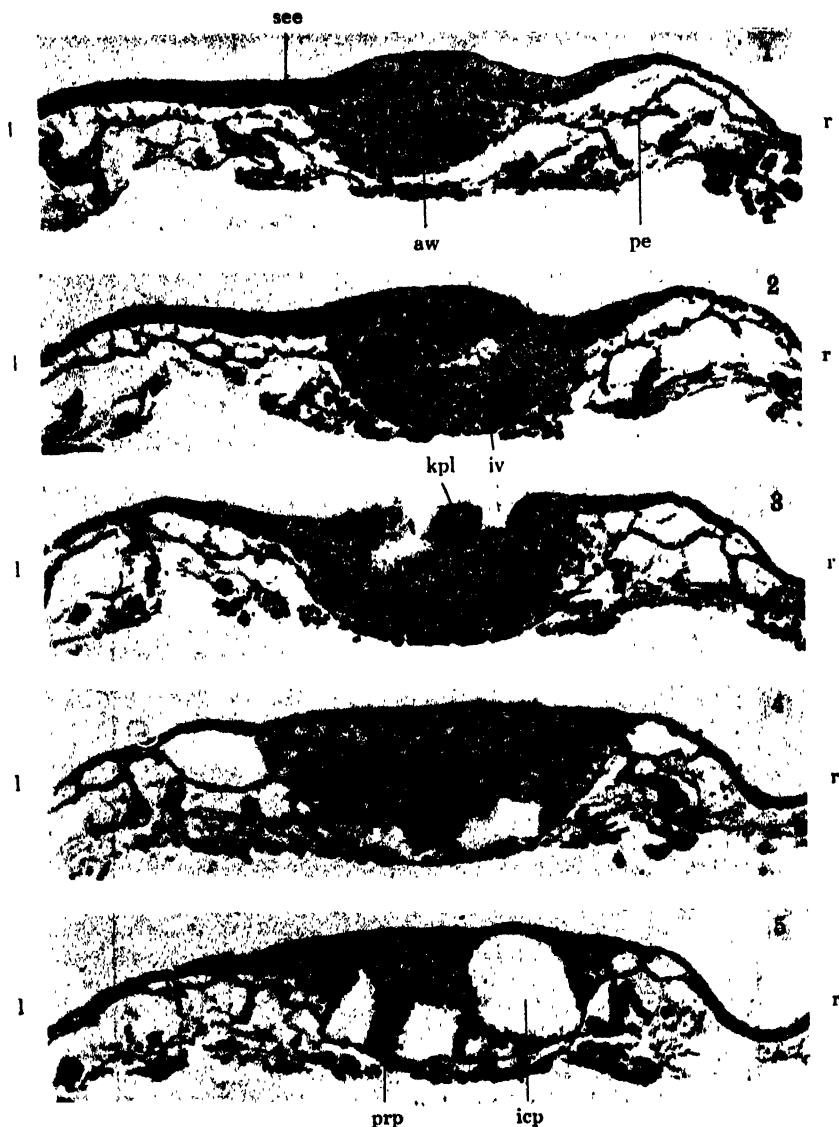


Fig. 9. Photomicrograph of transverse sections of specimen in Stadium II of nearly same age as that in Fig. 8, in order of 1-5 from anterior to posterior, showing dorso-ventrally flattened invagination cavity in 2, knob in region of posterior blastoporic lip in 3, and large intercellular cavities in primitive plate, which are most remarkable in 4 and 5.  $\times 80$ . *aw* anterior wall of invagination cavity, *icp* isolated parablasic cell, *iv* invagination cavity, *kpl* knob of posterior lip, *l* left side, *pe* primary endoderm, *prp* primitiv plate, *r* right side, *see* ectoderm of embryonic shield.

ly called the 'anterior wall' is more appropriately termed the 'dorsal wall' of the invagination cavity.

The invagination cavity broadens laterally (Fig. 9<sub>2</sub>, and IX<sub>4 and 5</sub> in Pl. X), and is barely overlapped by the anterior lip, which consists of two layers, viz. the outer, thinner ectoderm and the inner, thicker secondary endoderm (Fig. 7). On the rim of the anterior lip, both layers unite with each other, and their continuation forms the roof of the invagination cavity (IX<sub>4 and 5</sub> in Pl. X). The ectodermal epithelium loses its columnar arrangement of cells in the united region of both layers, and reveals an active proliferation directed anteriorly (Fig. 7). It has been ascertained that migration of cells from the ectoderm to the secondary endoderm occurs at this turned-in region at the top of the anterior lip. In this stage, the columnar arrangement of cells on the walls of the invagination cavity is restricted only to the dorsal and anterior parts, though it is more indistinct than that in earlier stages.

Several intercellular spaces are often found in the cell-mass of the primitive plate, and, in one specimen (Ser. 17), they were extraordinarily large, forming cavities (Fig. 9<sub>4 and 5</sub>).

The primary endoderm coheres to the underface of the plate substance, particularly in its anterior part. In the posterior region, however, more or less, the coherence is so loose, that irregularly slit-like spaces are found between the two (Fig. 7, and IX<sub>11-13</sub> in Pl. X). Along the anterior extremity of the wall of the invagination cavity, the primary endodermal layer creeps dorsally in contact with its surface, and it seems as if it were the continuation of cells situated on the outer surface of the dorsal wall (Figs. 6, 7 and 8). In the region anterior to this, amoeboid parablasic cells, scattered in the subgerminal fluid cavity, cohere to the primary endoderm, and frequently form an irregular honey-combed structure (Fig. 8).

The relation between the ectoderm and the primary endoderm is still intimate at the annular zone, as seen in earlier stages (V in Pl. IX).

In specimens at a little later stage, the contour of the invagination cavity shows no remarkable changes, but the forward encroachment of the anterior extremity of the anterior wall is very conspicuous, and shows a typical head process as a triangular prominence in a median longitudinal section. This process is encroaching in contact with the underlying endodermal layer, which is not already a unicellular, but, partially, and very irregularly, a layer of two- or three-cell thickness, owing to the addition of subjacent parablasic cells. Consequently, the differentiation into the material of the head process and that of the primary endoderm is indistinct,

and it is difficult to point out the anterior limit of the head process.

Cells, forming walls of the invagination cavity, no longer show a columnar arrangement. Accordingly, the surface of the walls, facing the cavity, becomes somewhat irregular.

The cells in the upper region of the primitive plate are associated loosely, and those in the deeper region comparatively compactly. The mitotic figures in the region near the posterior margin of the plate are very remarkable, and show the participation of these cells in the formation of the peristomial mesoderm.

In this stage, the peristomial mesoderm makes its appearance for the first time, and the distinctness between the peristomial mesoderm and the superficial ectoderm is clear at the posterior margin of the primitive plate, particularly at the margins postero-lateral to the plate.

### STADIUM III

As the development goes on, the invagination cavity deepens rapidly in the anterior direction (Figs. 10 and 11), and finally assumes a horizontal

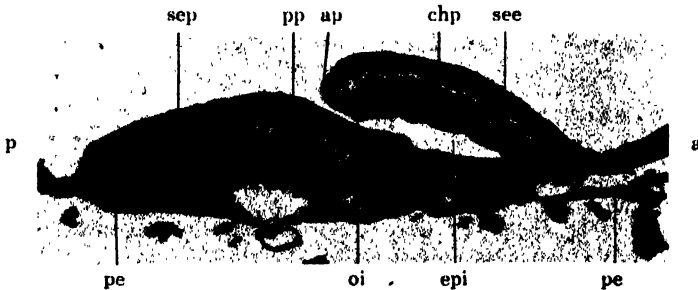


Fig. 10. Photomicrograph of median longitudinal section of specimen in Stadium III, showing two parts of invagination cavity and chorda primordium originating from dorsal wall of invagination cavity. Dorsal and ventral walls of invagination cavity are almost the same in thickness. Ventral face of primitive plate becomes flat owing to its antero-posterior extension.  $\times 80$ . *a* anterior, *ap* anterior lip, *chp* chorda primordium, *epi* expanded portion of invagination cavity, *oi* narrow outlet of invagination cavity, *pe* primary endoderm, *pp* posterior lip, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.

position, as a whole (Fig. 12.). The invagination cavity in the present stadium, when viewed from the dorsal, is an elongated triangle directed anteriorly with an anterior extremity, which ends more or less pointedly ( $X_1$  in Pl. X). In transverse sections, the middle part of the cavity is spindle-shaped, being expanded laterally ( $X_2$  in Pl. X), and the hinder-

most part, or the region immediately anterior to the outer opening, forms a slit-like space, being flattened dorso-ventrally ( $X_6$  in Pl. X). In a median longitudinal section, the posterior part of the cavity is narrow, forming a slit-like space, and is distinguished from the middle, dorso-ventrally expanded part (Figs. 10, 11 and 12<sub>1 and 2</sub>). The roof of the cavity

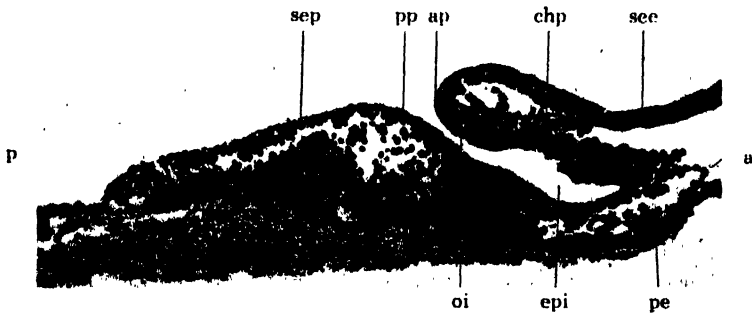


Fig. 11. Photomicrograph of median longitudinal section of specimen of nearly the same age as that in Fig. 10, showing somewhat dorso-anteriorly directed, anterior extremity of invagination cavity and loosely associated cells in ventral wall of invagination cavity.  $\times 80$ . Abbreviations as in Fig. 10.

consists of a thinner, superficial ectoderm and a thicker turned-in endoderm ( $X_5$  in Pl. X), which now forms the chorda dorsalis (Figs. 10–12<sub>1</sub>).

The anterior lip somewhat overhangs the posterior lip, so that, hereafter, they may be called, respectively, the dorsal and the ventral lips. This condition of the blastopore, just stated, represents a transitional stage from an archistome to a prostome.

The surface of the primitive plate is now slanting posteriorly, and its ventral face becomes more flattened. On the boundary between the primitive plate and the annular zone, there exists a shallow groove, which has been making its appearance from a much earlier stage (Figs. 10–12<sub>1</sub>). Owing to the downgrowth of the anterior part of the primitive plate caused by the deepening of the invagination cavity and to the formation of the peristomial mesoderm in the posterior region of the plate, the plate itself becomes flat and elongated antero-posteriorly. The lateral extension of the rudimentary peritomeal mesoderm is also fairly remarkable, i. e. the distance between the ventral lip and the posterior margin of the plate is much less than the breadth of the plate at the level of the ventral lip. For instance, the measurements of three typical specimens are given here: viz. the distance and the breadth was, respectively, 0.42 mm., 1.05 mm. in a specimen numbered Ser. 18; 0.55 mm., 1.04 mm. in Ser. 8; and 0.53 mm.,

1.02 mm. in Ser. 19. In the present stage, the cells near the surface of the primitive plate proliferate towards the inner zone. Such a figure is most noticeable, particularly in the posterior half of the plate. It may,

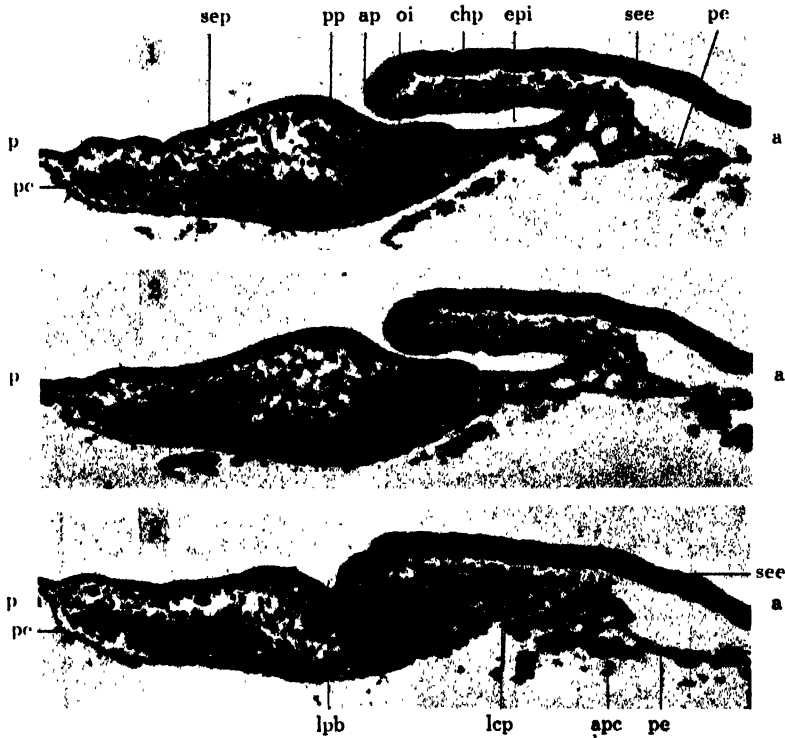


Fig. 12. Photomicrograph of longitudinal sections of specimen in Stadium III, later than that in Fig. 11, in order of 1-3 from median to right, showing fully grown invagination cavity lying horizontally as a whole.  $\times 80$ . In 1 (median), chorda primordium is in course of establishment, and tissue at anterior extremity of wall of invagination cavity forms meshes of network. In 2, floor of invagination cavity is recognizable as a double layer. In 3, laterally extended tissue of chorda primordium is obvious. *a* anterior, *ap* anterior lip, *apc* aggregation of parablastic cells, *chp* chorda primordium, *epi* expanded portion of invagination cavity, *lcp* lateral margin of chorda primordium, *lpb* lateral portion of blastopore, *oi* narrow outlet of invagination cavity, *p* posterior, *pe* primary endoderm, *pp* posterior lip, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.

therefore, be suggested that these proliferated cells participate in the formation of the peristomial mesoderm.

In the ventral part of the anterior extremity of the wall of the invagination cavity, cells are most loosely associated, and form irregular inter-

cellular cavities showing a honey-combed structure (Fig. 12<sub>1</sub>). Some of the cavities communicate with the subgerminal fluid cavity, breaking through the underlying primary endoderm (Fig. 12<sub>1 and 2</sub>).

With the progress of development, the invagination cavity attains its full length, indicating the stage immediately before the breaking through of the invagination cavity into the subgerminal cavity below. Simultaneously, the anterior part of the invagination cavity, which has been shown as a triangle in a surface view, expands more laterally (Fig. 14<sub>2</sub>), and the secondary endodermal layer, which has been forming the upper part of the floor of the invagination cavity, becomes thinner, rapidly combining more steadily with the primary endodermal layer than is the case in earlier stages (Fig. 14<sub>1</sub>). The conditions, just mentioned, give marked characteristics to this stadium.

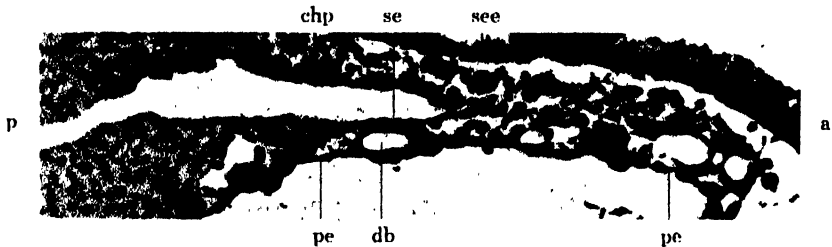


Fig. 13. Photomicrograph of median longitudinal section of specimen in Stadium III, slightly later than that shown in Fig. 12, showing part of floor of invagination cavity consisting of double layer.  $\times 160$ . *a* anterior, *chp* chorda primordium, *db* double layer consisting of primary and secondary endoderms, *p* posterior, *pe* primary endoderm, *se* secondary endoderm, *see* ectoderm of embryonic shield.

Irregular intercellular cavities, which have been formed in the ventral region of the anterior wall of the invagination cavity, now become more conspicuous and form meshes of network (Fig. 13). Cells in the floor, i. e. in the region somewhat posterior to, and, particularly, postero-lateral to, the region just noted, tend to show a similar structure (Figs. 12<sub>1 and 2</sub>, 13 and 14<sub>2 and 3</sub>). Some of the cavities communicate with the subgerminal cavity, as have been found in the anterior wall of the invagination cavity in earlier stages (Fig. 14<sub>1-3</sub>). It is noteworthy, that the floor of the invagination cavity is represented by a sheet, which consists, temporarily, of two cell-layers, viz. the primary and secondary endoderms. This structure is most distinct, when the intercellular cavities appear in this region (Fig. 13), but soon disappears. When the retirement of the region here described takes place, the area of the floor becomes occupied, partially, by a some-



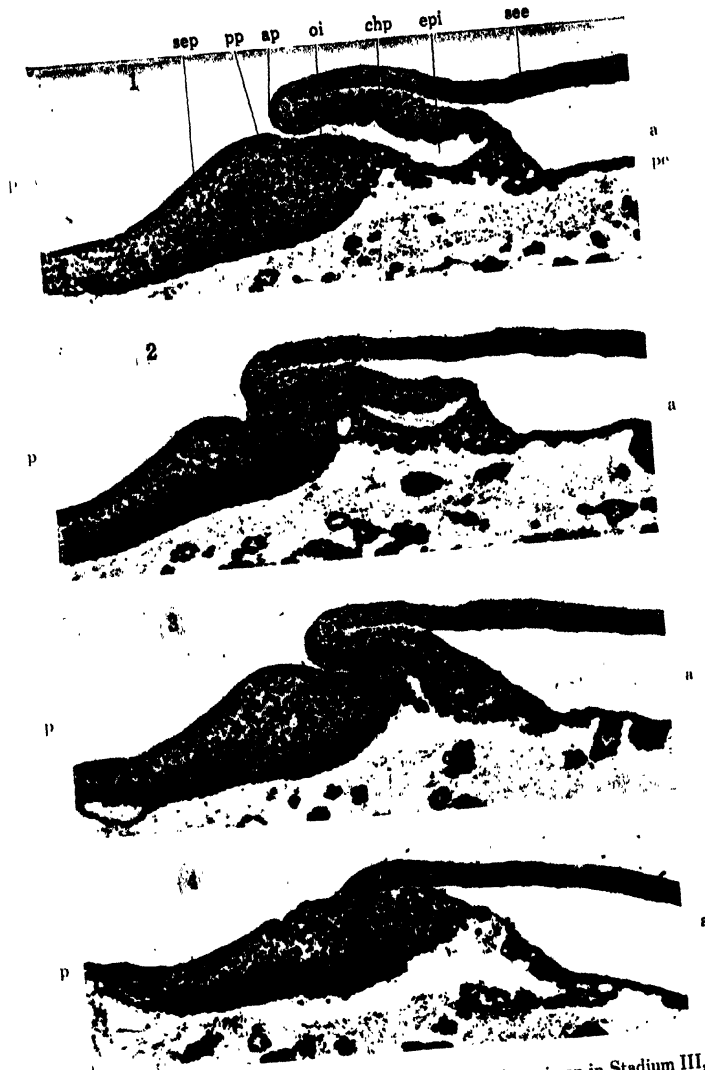


Fig. 14. Photomicrograph of longitudinal sections of specimen in Stadium III, later than that seen in Fig. 13, in order of 1-4 from median to right, showing median floor of invagination cavity consisting of irregular layer in 1 and lateral extension of anterior portion of invagination cavity in 2.  $\times 80$ . *a* anterior, *ap* anterior lip, *chp* chorda primordium, *epi* expanded portion of invagination cavity, *oi* narrow outlet of invagination cavity, *p* posterior, *pe* primary endoderm, *pp* posterior lip, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.

what irregular sheet consisting of both layers. The irregularity in this sheet, frequently, strengthens in degree, owing to the addition of subjacent parabolastic cells (Fig. 14<sub>1</sub>). The condition, above-stated, is restricted only in the floor region of the invagination cavity, and its anterior extremity is, more or less, raised dorsally, showing the condition that has been observed in earlier stages.

Owing to the rapid encroachment of the tip of the invagination cavity, its anterior extremity becomes bounded by a layer of two- or three-cell thickness from the intervening cavity between the superficial ectoderm and the primary endoderm, as clearly seen in a further developed specimen (Fig. 14<sub>1</sub> and 37). The formation of intercellular cavities in the anterior wall becomes gradually more prominent, and, finally, some of them communicate with the intervenient cavity anterior to them.

By this stage, the chorda primordium has readily attained the posterior half of the embryonic shield, and the cells constituting it are arranged very loosely, presenting a characteristic appearance in sections (Fig. 13).

On the rim of the dorsal lip, cells are actively proliferating forwards. In a rather more developed specimen, one of the most characterized features is a marked increment in the number of cells, owing to an active cell-division at the turned-in rim of the secondary endoderm, showing the presence of a definite intumescence, which is most prominent particularly on the median longitudinal line. No ectodermal elements participate in the formation of the intumescence. The corresponding ventral wall sinks responding to this thickening, and forms a well-marked depression. This is a temporary condition, which, however, remains the same until the stage at which the breaking through occurs.

In connection with the rapid formation of the peristomial mesoderm, the primitive plate extends posteriorly and postero-laterally much more than in earlier stages, and flattenes, slanting smoothly towards the posterior. On the hinder margin of the plate, there is now no furrow, such as has been recognized in earlier stages; accordingly, the boundary between the primitive plate and the annular zone becomes quite indistinct. In this region, the formation of the peristomial mesoderm from the cells of the primitive plate is most active, and encroaches posteriorly and postero-laterally between the superficial ectoderm and the underlying endoderm. The mesodermal layer is distinctly separated from the primary endoderm, but adheres to the superficial ectoderm. From precise observations of the appearance of the protoplasmic processes and the wedging of cells, it is suggested that an addition of cells from the ectoderm occurs. On the

contrary, there is no connection between the mesoderm and the primary endoderm.

The parablastic cells are still forming aggregations in the subgerminal cavity. Owing to the addition of some of these cells, the primary endoderm underneath the embryonic shield is not already a perfectly unicellular, but forms, partially, a very irregular layer of two- or three-cell thickness, the coherence of more subjacent cells being observed.

#### STADIUM IV

This stadium includes the stages of the breaking through of the invagination cavity into the subgerminal cavity.

The chorda primordium, or the original dorsal wall of the turned-in endoderm, extends through nearly half the length of the embryonic shield, and the floor of the invagination cavity, which is just in the course of breaking through, attains about half the total length of the cavity (VIII, in Pl. IX).

The endodermal sheet, which has been shown previously as a double layer intervening between the invagination cavity and the subgerminal cavity, becomes thinner until both cavities communicate with each other. It is of great interest to pursue the factors causing the breaking through. The disappearance of the cells, forming the endodermal sheet, is caused principally by the lateral and anterior retirement due to a rapid growth of the chorda primordium, and, partly, by the retreatment of the anterior boundary of the primitive plate. In this case, the endodermal sheet is simply drawn circumferentially, but some cells frequently remain on the level of the area of breaking through, this depending on the condition that the cells, constituting this sheet, are not arranged uniformly, as mentioned previously (Figs. 15, and 16). In this region, besides these cells, the subjacent parablastic cells are frequently found scattered, either isolated or in aggregated groups. Both kinds of cells are easily distinguishable, according to the large size and well-staining property of the cytoplasm in the subjacent cells (Fig. 15<sub>1</sub> and 2). These conditions last in the succeeding stages.

As, at first, the breaking through takes place generally in the central depressed portion in the floor of the invagination cavity, shelves of cells, directed anteriorly and posteriorly, remain on the level of breaking through, as shown in the median longitudinal sections (Figs. 15, and 16, and VIII, in Pl. IX). Both lateral portions of the broken floor still consist of a

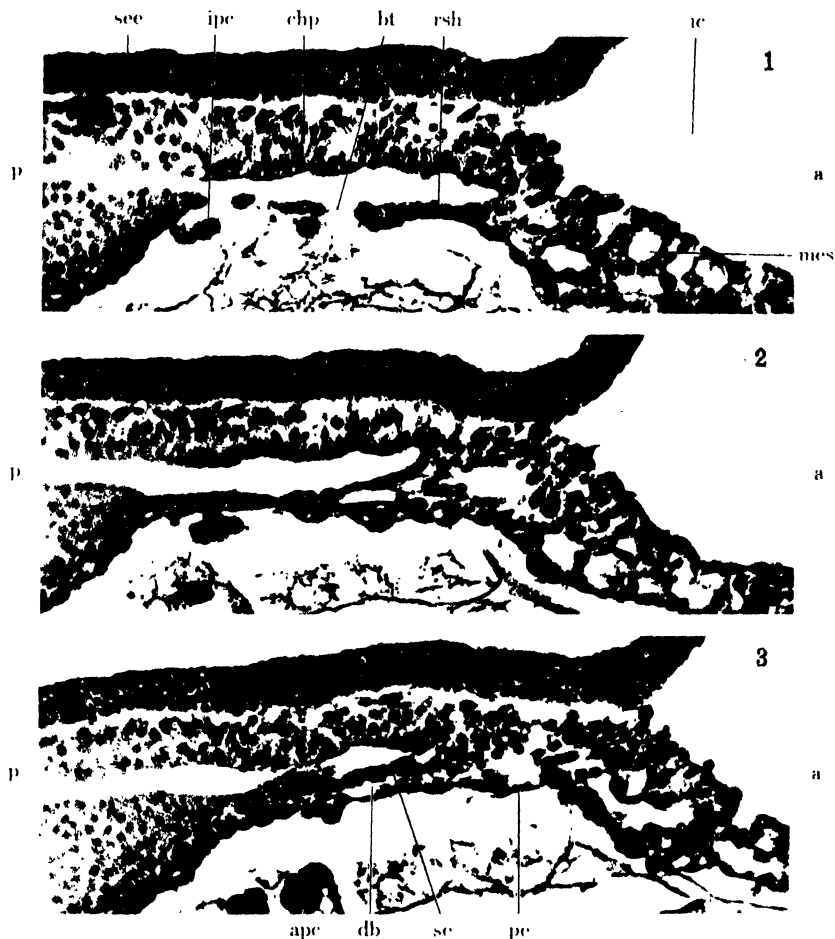


Fig. 15. Photomicrograph of longitudinal sections of specimen in Stadium IV, in order of 1-3 from median to lateral, showing commencement of breaking through and remaining shelf in 1, single-layered floor in 2, and double-layered floor in 3.  $\times 160$ . *a* anterior, *apc* aggregation of parablastic cells, *bt* breaking through in floor of invagination cavity, *chp* chorda primordium, *db* double-layered floor of invagination cavity, *ic* interventive cavity between ectoderm and endoderm, *ipc* isolated parablastic cell, *mes* meshes of network, *p* posterior, *pe* primary endoderm, *rsh* remaining shelf of floor of invagination cavity, *se* secondary endoderm, *sec* ectoderm of embryonic shield.

single layer, and going further laterally, for this single layer is substituted a double layer, as has been observed in the median sections of earlier specimen (Fig. 15<sub>1-3</sub>). The remaining cell-shelves become completely obliterated in the immediately succeeding stage. When the thinned floor of

the invagination cavity disappears, or the movement of breaking through is completed, the blastoporic passage, or Kupfferian canal, makes its appearance for the first time.

Cells, constituting the original anterior wall of the invagination cavity, encroach more anteriorly, in contact with the surface of the primary endoderm, and form a number of irregular intercellular cavities in its structure, presenting a remarkably peculiar appearance (Fig. 15<sub>1, 3</sub>, and VIII<sub>1 and 2</sub> in Pl. IX). Some of the intercellular cavities, situated comparatively posteriorly, often communicate with the invagination cavity and with the subgerminal cavity (VIII<sub>2</sub> in Pl. IX); others are open to the blastocoelic cavity, which is anterior to the intercellular cavities, and which intervenes between the ectoderm and the primary endoderm (Fig. 15<sub>1 and 3</sub>).

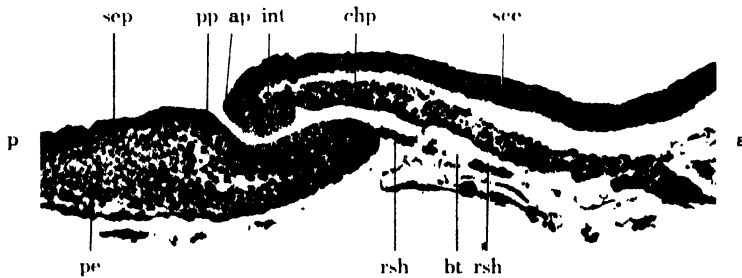


Fig. 16. Photomicrograph of median longitudinal section of specimen in Stadium IV, later than that in Fig. 15, showing much elongated chorda primordium and real state of breaking through of floor of invagination cavity. Intumescence on ventral margin of anterior lip, and remaining shelves are seen also.  $\times 80$ . *a* anterior, *ap* anterior lip, *bt* breaking through of floor of invagination cavity, *chp* chorda primordium, *int* intumescence at anterior lip, *p* posterior, *pe* primary endoderm, *pp* posterior lip, *rsh* remaining shelves of floor of invagination cavity, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.

Finally, the primary and secondary endoderms in this region merge into each other, so that, particularly in the anterior portion, the differentiation into both endoderms becomes more or less indistinct. But, as noted before, the secondary endoderm is represented generally by a thicker layer of loosely associated cells, containing many intercellular vacuoles, while the primary endoderm is represented by a thinner layer, partially, of two- or three-cell thickness.

In the succeeding stages, the primary endoderm might have appeared as an anterior continuation of the secondary endoderm in the median section (Fig. 16). But this is not the case. The anterior extremity of the secondary endoderm, which is represented by that of chorda primor-

dium, is scarcely recognizable, and the posterior limit of the primary endoderm in this region is never indicated by the anterior extremity of the chorda primordium, because the occurrence of the former ought to be more posterior. This fact may be comprehended more clearly if the positional relation between the anterior extremity of the secondary endoderm and the region of breaking through has been understood in a rather earlier stage.

Resulting from these conditions, the blastocoelic space, intervening between the ectoderm and the endoderm, becomes obliterated by the forward encroachment, by degrees, of the chorda primordium, beginning at its median posterior part.

The ventral surface of the chorda primordium, facing the Kupfferian canal, consists of compactly associated cells, but, on the contrary, the dorsal surface, facing the ectodermal layer, consists of very loosely associated cells, from each of which an irregular protoplasmic process projects towards the ectoderm (Fig. 15<sub>1</sub> and 2, and VIII<sub>1-4</sub> in Pl. IX).

On the rim of the dorsal blastoporic lip, the turned-in endoderm shows a notable intumescence, apparently owing to active cell-division in this portion. This intumescence is that, which has been already observed in earlier stages, but, in Stadium IV, it is most pronounced (VIII<sub>1</sub> in Pl. IX). In a later stage of this stadium, the intumescence gradually disappears (Fig. 16). The dorsal lip is much raised, overlapping the ventral lip completely. This condition indicates, typically, what is called the prostome.

The primitive plate is much flattened and remarkably expanded. The formation of the peristomial mesoderm is also very conspicuous, owing to the posteriorly directed proliferation of cells in the undifferentiated portion of the primitive plate and to the addition of a small number of cells from the superficial ectoderm. The mesodermal cells encroach further posteriorly to a considerable extent, and form a definite sheet terminating freely in a pointed end. The primary endodermal layer is well demarcated in the mesodermal region and in the posterior region of the primitive plate. In the anterior region, however, the primary endoderm coheres closely to the cell-mass of the primitive plate, and, finally, its distinctness on the foremost boundary is no longer visible (Fig. 16, and VIII<sub>1</sub> in Pl. IX).

#### STADIUM V

This stadium, which includes all the serial stages later than the completion of the Kupfferian canal, is characterized by the alteration of the

form of the blastoporic passage, by the establishment of the chorda primordium, and by the formation of the gastral mesoderm.

The Kupfferian canal becomes more oblique and shorter, owing to the receding back of the anterior margin of the primitive plate, which limits the area broken through. Thus, this is a short oblique canal with a posterior (dorsal, outer) and an anterior (ventral, inner) opening, by which the outside of the embryo and the subgerminal fluid cavity respectively communicate (XI, in Pl. X). But, a little later, the anterior opening narrows, so that the canal has a funnel shape being flattened dorso-ventrally, with its narrow end anteriorly directed (XI, in Pl. X).

The parablasic cells often remain in the subgerminal fluid cavity in the region of the anterior opening, but, some of them, which are anterior in position, are also in close contiguity with the underface of the primary endoderm. It is worthy of note that this structure may easily be mistaken for a remaining anterior shelf (XI<sub>1, 3</sub> and 4 in Pl. X).

The remarkable intumescence on the rim of the dorsal lip disappears with the completion of the Kupfferian canal. The present writer is not able, at present, to explain the significance of this thickening. When the intumescence disappears, the chorda primordium, between the rim of the dorsal lip and the region slightly anterior to the ventral opening, shows a uniform thickness. The thickness of the superficial ectoderm, which has remained almost the same from an earlier to this stage, tends to increase, but, on the contrary, the loosely structured chorda primordium becomes remarkably compact and comparatively thin. Consequently, in the region just noted, both layers, the superficial ectoderm and the chorda primordium, are now nearly the same in thickness (XI, in Pl. X). Further anteriorly, the differentiation, between the chorda primordium and the primary endoderm, becomes very indistinct, so that there exists now a layer, parallel to and beneath the ectoderm, there being a space of about a six's breadth between them. This parallel, underlying layer is by degrees consistently thinner anteriorly (XI, in Pl. X).

The primitive plate is gradually diminishing in depth to its anterior part, and combines more continuously with the underlying endoderm than it does in earlier stages. In regard of these conditions, the distinct appearance of the primary endoderm is detectable only in the posterior half of the primitive plate and in the lateral regions of the shield, where the chorda primordium does not spread (XI<sub>1</sub> and 5-6 in Pl. X). The primitive plate becomes much flattened, slanting further posteriorly as a whole. The rapid backward extension of the plate is related closely to

the formation of the peristomial mesoderm, owing to a conspicuous migrating activity of the undifferentiated cell-mass. The developmental degree of the peristomial mesoderm differs much even in individuals at the same stage, and, in a typical specimen (Ser. 37), its median length measured 1.4 mm. and reached two-thirds of the length of the embryonic shield.

In specimens in this stadium, there is found an active cell-proliferation, directed antero-laterally at both regions lateral to the posterior opening of the Kupfferian canal. This cell-proliferation is the first appearance of the gastral mesoderm. Thus, the formation of the gastral mesoderm is delayed more than the establishment of the chorda primordium. As the lateral and antero-lateral extensions of the gastral mesoderm proceed, in both proximal parts, the chorda primordium comes in contact with the gastral mesoderm, and both elements merge into each other, with an addition of cells from the pre-existing chorda primordium. This condition is most remarkable in the region near the Kupfferian canal, and by degrees becomes anteriorly slighter. The proliferated cells are more loosely associated when going further from the zone of differentiation, and show typical mesodermal characters, each cell being round-shaped ( $XI_5$  in Pl. X). On a transversal line through the posterior opening of the Kupfferian canal, the gastral mesoderm comes in contact with the lateral extension of the peristomial mesoderm, which is already formed ( $XI_{11-12}$  in Pl. X). That is to say, the gastral mesoderm represents both antero-laterally extended portions of the peristomial mesoderm as a whole. The peristomial mesoderm extends caudally, attached to the superficial ectoderm, while the gastral mesoderm creeps, antero-laterally, from the regions of its origination, along the upper face of the primary endoderm, with a slit-like space between it and the ectoderm. So, the positional substitution of both mesoderms clearly gives rise, in the region immediately lateral to the posterior opening of the Kupfferian canal ( $XI_{1-6}$  in Pl. X). The margins of the extended gastral mesoderm terminate in a somewhat blunt end ( $XI_{7 \text{ and } 8}$  in Pl. X).

The latest stage at the disposal of the present writer is characterized by prosperous wing-like extensions of the gastral mesoderm. The lateral and antero-lateral extensions of the mesoderm are very rapid in Stadium V. Consequently, the anterior extremity of the chorda primordium is situated along the median line more posteriorly to that of the gastral mesoderm in either lateral portion of the embryonic shield. The gastral mesoderm becomes separated more or less distinctly from the primary endoderm, and forms a sheet of cells. The anterior margin of the gastral mesoderm thickens, forming a small cell-aggregation, and coheres with the



superficial ectoderm more closely than elsewhere. This remarkable extension of the gastral mesoderm in specimens belonging to this stage has been termed 'Mesodermflügel' by BALLOWITZ in the case of *Tropidonotus natrix* BOIE.

The shape of the posterior opening of the Kupfferian canal represents a transitional condition between a prostome and a metastome.

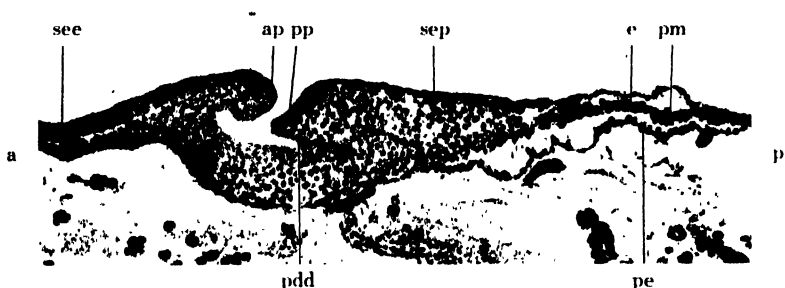


Fig. 17. Photomicrograph of longitudinal section, slightly lateral to median, of specimen in Stadium V, showing peculiar inlet in region of posterior lip. Laterally extended posterior portion of Kupfferian canal, and peristomial mesoderm insinuated posteriorly between ectoderm and endoderm are also shown.  $\times 80$ . *a* anterior, *ap* anterior lip, *e* ectoderm of annular zone, *p* posterior, *pdd* posteriorly directed inlet of Kupfferian canal, *pe* primary endoderm, *pm* peristomial mesoderm, *pp* posterior lip, *see* ectoderm of embryonic shield, *sep* superficial ectoderm of primitive plate.

In some specimens (Sers. 26, 30 and 37), there was found on the outer surface corresponding to the part slightly on the inner side of the original posterior lip, a peculiar backward inlet, the walls of which consist of a compact cell-layer similar to that on the surface of the primitive plate (Fig. 17, and XI<sub>2</sub> in Pl. X). This inlet resembles that found already by KUPFFER (1882) in *Lacerta viridis*, by WENCKEBACH (1891) in *Lacerta agilis*, and by INUKAI (1927) in *Takydromus tachydromoides*. More examples are, however, necessary in order to accomplish the investigation in regard to this inlet.

## CONCLUSIVE REMARKS AND COMPARISON WITH RESULTS PREVIOUSLY OBTAINED

### I. SUBGERMINAL FLUID CAVITY AND ITS SIGNIFICANCE

In the Ophidia, as the present writer has already noted in regard to its early developmental stages, an extraordinarily spacious subgerminal fluid cavity is present underneath the blastoderm. In this cavity, a quantity

of suffused subgerminal fluid and a great number of free cells are recognizable as the contents. From the fact that such a spacious subgerminal cavity is never found in other footed reptiles, it is obvious that the occurrence of this extensive cavity is caused by the soft property of the shell-membranes, by the scantiness in albumen, and by the morphological and ecological conditions in the case of maternal snakes.

## II. ORIGIN OF PARABLASTIC CELLS

It is also confirmed that many various parablasic cells scattered in the subgerminal cavity are produced mainly by proliferation in the region of the yolk surface and in a few cases of the circumferential germ wall. Such cells in snakes have already been described by KUPFFER (1882), WILL (1899), GERHARDT and O. HERTWIG (1901), and by BALLOWITZ (1901). The present writer, also, investigated the origin of these cells, and ascertained that they issue from the simple upward isolation of cells, which are scattered in, or of meroblastic nuclei, which are embedded in the yolk matter, after the establishment of the blastoderm.

## III. KINDS OF THE PARABLASTIC CELLS

Each cell, which is situated in the upper zone of the subgerminal cavity and contains a comparatively distinct nucleus, and which resembles the cells of the incomplete primary endodermal layer at the time, indicates that the stage of its proliferation is earlier than that of each cell, which is situated in the lower zone of the cavity, on account of their positional and morphological relations. It is, therefore, affirmed that the cells, which are situated more deeply or are scattered nearer the yolk surface, are formed developmentally later than the upper ones.

KUPFFER states that "Die am tiefsten sich findenden Elemente erscheinen, nach Anwendung des Eingangs erwähnten Färbeverfahrens, als blasse feingranulierte Kugeln ohne wahre Kerne, aber versehen mit 1-2 anseheinend homogenen, intensiv gefärbten Kernkörperchen." The present writer, however, in no case found spheres corresponding to the "Kugel" of KUPFFER. Any sphere which has no nucleus is not to be considered a real cell, but corresponds probably to the yolk-mass observed by the present writer, isolated from the yolk surface together with the proliferated parablasic cells.

It is probably a misinterpretation to conclude that the dissimilarity in feature between upper and lower cells, due to their positional difference,

is caused by the metamorphosis of the cells themselves. It is rather to be presumed that the dissimilarity is caused by the difference in the periods of their origination. The upper cells, which are proliferated earlier, present a resemblance in feature to the cells, which are produced in the course of the constitution of the primary endoderm. As the proliferation of the lower parablasic cells becomes feebler with the progress of development, these cells, which originate later than the upper parablasic cells, and which are not formed already in the deeper part, seem to show a degenerative condition. Accordingly, the presence of the cells is doubtful, on which KUPFFER notes that "diese folgen gegen die Oberfläche hin gleichfalls kugelige Elemente (a kind of parablasic cells according to him) von annähernd derselben Grösse, die in toto lebhaft gefärbt und stärker granuliert erscheinen, aber weder Kerne noch Kernkörperchen aufweisen." As to such cells, as KUPFFER describes, the present writer thinks presumably that the nucleus and the nucleolus were overlooked in consequence of the strong staining property of the cell-contents.

#### IV. ARRANGEMENT OF THE PARABLASTIC CELLS

According to GERHARDT, these free parablasic cells assemble at first in threads, starting at the roof of the subgerminal cavity, upwards, and reaching to its floor, downwards. The threads of these cells are directed vertically at the beginning of their formation, but, generally, become irregular being associated with other threads horizontal to the roof of the cavity, and, the horizontal threads becoming finally predominant. Notwithstanding this, in the material under investigation, the present writer was not able, thoroughly, to observe such comparatively regular, morphological changes. Although GERHARDT did not note fully regarding the cells, corresponding to the deeper cells found by the present writer, his cells appear to belong to the writer's upper cells, and these upper cells aggregate from the first in a very irregular manner, without showing any resemblance to the process of development given by GERHARDT. The condition of the upper cells, which remained slightly in the region of the subgerminal cavity, is confirmed even in a much later stage, i. e. after the completion of the Kupfferian canal, rather in agreement with the description of WILL.

#### V. CONNECTION BETWEEN, AND THE ALTERATION OF THE FORM OF, THE PARABLASTIC CELLS

GERHARDT speaks of fine protoplasmic processes connecting the parablasic cells. Allied structures were also found in the specimens of the

present writer, but they are apparently structures caused by coagulation of the subgerminal fluid due to hardening. According to him, "Die dotterhaltigen Zellen verlieren dabei den Dotter mehr und mehr, so dass um den Zellkern schliesslich nur ein ganz schmaler Protoplasmahof übrig bleibt." The present writer objects, however, to his interpretation in regard to this phenomenon, given in the above statement.

## VI. GROWTH OF THE AGGREGATION OF PARABLASTIC CELLS

BALLOWITZ observes mitotic figures in the cells of aggregations which are situated near the primary endoderm. GERHARDT also mentions that the thread of cells grows by division of the cells constituting the thread itself and by the addition of the parablasic cells from the lower part of the cavity. Notwithstanding this, the present writer did not observe any mitotic figures in the subgerminal cavity. Regarding the growth of the cell-aggregated thread, he is of opinion that it is caused only by the annexation of free parablasic cells, which are gradually proliferated upwards from the yolk-surface by the so-called 'Nachfurchung.'

## VII. PRIMARY ENDODERM

Underneath the blastoderm, the parablasic cells, in isolated and aggregated conditions, connect with the underface of unicellular primary endodermal layer, which is not yet completely formed. This layer is one of the most marked characteristics in reptiles, and is variously termed by many preceding investigators: viz. 'Entoderm' by BALLOWITZ, 'Lecithophor' by VAN BENEDEN, 'innere Keimblatt,' 'primäre Entoderm,' or 'Darmdrüsenblatt' by GERHARDT, 'Enteroderm' by GÖTTE, 'untere Keimblatt' or 'innere Keimblatt' by O. HERTWIG, 'cenogenetic hypoblast' by HUBRECHT, 'secundäre Entoderm,' 'Dotterblatt,' or 'Paraderm' by KUPFFER, 'Paraderm' by MEHNERT, 'cenogenetische Entoderm' by WENCKEBACH, 'sekundäre Entoderm' by WILL, etc. From the phylogenetic view, the endoderm is of secondary formation. This layer is, however, already in the course of establishment, before the first appearance of the turned-in endoderm caused by the invagination in the anterior part of the primitive plate. From the ontogenetic view, therefore, it is of primary formation. In the present paper, the present writer prefers consistently the term 'primary endoderm' for the endoderm under discussion. For the same reason, the 'primäre Entoderm' of GERHARDT does not correspond to that of KUPFFER and of WILL, but to their 'secundäre Entoderm.'

## VIII. ORIGIN OF THE PRIMARY ENDODERM

Concerning the origin of the primary endoderm, the GERHARDT's view is in substance as follows: generally before the primary endoderm forms, a distinct cell-anlage of the primitive plate has already been established, and, from the primary endoderm, fine connecting processes reach, partially, to the undifferentiated ectodermal cell-mass of the primitive plate, into which the endodermal cells migrate, and both materials are combined with each other. Underneath this region, a single layer of the primary endoderm, which is scarcely recognizable, but, as a whole, is a new continuous substance, makes its appearance. This primary endoderm is more belated in process of formation and is more incomplete in structure in the further anterior region than in that of the primitive plate. From these two facts, it may be assumed that the primary endoderm originates from the primitive plate and by degrees extends anteriorly between the ectodermal and yolk substances. GERHARDT averred, consequently, that a somewhat characteristic process of invagination is reviewed in regard to the method of the formation of the primary endoderm.

Notwithstanding this, the present writer obtained quite a different result in his observation of the origin of this endoderm. Underneath the primitive plate, there has already formed a single endodermal layer of loosely associated cells, which is discriminated only by careful observation, and, between the cell-mass of the primitive plate and the underlying endoderm, no such migrations of cells, as GERHARDT observed, were found. No difference was found between the cells constituting the primary endoderm and the parablasic cells scattered in the upper zone of the sub-germinal cavity. Moreover, in the region of combination of the parablasic cell-aggregations with the endoderm, the endodermal layer showed a notable irregularity, as already stated. It is, therefore, very clear that the primary endodermal cells are nothing but a kind of parablasic cells. This interpretation of the present writer's agrees well with that given by KUPFFER. In its peripheral region, the primary endodermal layer continuously passes into the germ wall, and the formation of the parablasic cells in this region is very active: consequently, the addition of the forms, the parablasic cells in large numbers to the primary endodermal layer are also clearly observable.

## IX. ORIGIN AND GROWTH OF THE PRIMITIVE PLATE

\* Various views as to the method of formation of the cell-mass of the

primitive plate have been hitherto held by many investigators. In the region of this plate, WILL finds that there is a migration of the subjacent endodermal cells to the cell-mass constituting the primitive plate, and remarks that the voluminal increase of the plate is caused not only by the increase in number of the original cells in the plate itself, but also largely by the addition and the immigration of subjacent cells. WILL's opinion inclines, consequently, to support the endodermal origin. On this point, GERHARDT and O. HERTWIG are of opinion that three germinal layers are connected with one another in the region of the primitive plate, and show an analogy with the primitive streak of the Mammalia and of the Aves. From this point of view, they argued that it is better to say that the primitive plate indicates a kind of neutral tissue belonging neither to the ectoderm nor the endoderm. This opinion is rightly derived from the result of the observation of the formation of the primary endoderm, mentioned above.

BALLOWITZ specifies three factors which participate in the formation of the primitive plate, viz. "1) eine Ein- und Anlagerung von Entodermzellen, 2) eine Verdickung des Schildepithels, 3) der Umstand, dass das Ektodermepithel an dieser Stelle seinen epithelialen Charakter verliert." That is to say, the thickening of the primitive plate arises at first from the active invasion of endodermal cells, and, then, these cells insert themselves in large numbers between the cells of the loosely associated cell-mass which are massed into a thick layer. It is his opinion that these cells are distinguishable according to their form, and, further that in this case the subjacent aggregation of cells participates in the invasion.

As mentioned above, BALLOWITZ affirms that the invasion of the endodermal cells takes place, at first, in the formation of the primitive plate, and then, the numerous mitotic division of the inserted cells occurs secondarily. BALLOWITZ, therefore, reaches the same conclusion as that of WILL. The observation result of the present writer differs, however, fundamentally, from the foregoing facts, i. e. when the primitive plate is in the course of formation, the endoderm is already present underneath the region of the primitive plate as a single incomplete layer. Cells of both substances cohere with each other, but they are clearly distinguishable according to their form and staining property. From this fact, it is obvious that such an invasion of the endodermal cells into the plate-substance, as found to be the case by BALLOWITZ, has never occurred in specimens of the present material in the earlier stage.

As regards the evidence above referred to, the growth of the cell-mass

in the primitive plate is caused apparently by the inward proliferation of the superficial ectodermal cells, after the deprivation of its epithelial character, and in this inner cell-mass itself, numerous mitotic figures are found, hence the successful activity of the cell-division in the inner cells is proved conclusively.

Regarding the increase in bulk in the cell-mass of the primitive plate, its underface, which was rather flat in earlier stages, becomes prominent downwards. As the result of the observation of the present writer shows, the substance of the primitive plate bulges downwards, and coheres more closely to the imperfect endodermal layer, which was already being established underneath the primitive plate. The present writer leans, consequently, to the opinion that the plate-substance originates, exclusively, from the ectoderm.

As to the origin of the thickening, the following factors may be mentioned:— viz. 1) disappearance of the columnar cell-arrangement in the superficial ectodermal layer, 2) cell-proliferation towards the inside in the ectoderm, and 3) active divisions of the proliferated cells.

#### X. INVASION BY THE SUBJACENT ENDODERMAL CELLS OF THE SUPERFICIAL ECTODERMAL LAYER

According to WILL, the distinct invasion of the subjacent cells so as to lodge among the cells constituting the superficial layer gives rise to the posterior portion of the primitive plate, to the circumferential annular zone and to the anterior region of the embryonic shield, and this superficial layer ought to be designated by the term 'Ectoderm,' when the invasion of these cells has ended in the further developed stage with a complete invagination cavity.

According to BALLOWITZ, the unevenness on the underface of the ectodermal epithelium in the region of the embryonic shield may be attributed to the addition due to the invasion of the endodermal cells, and, particularly, in the earlier stages, the endodermal cells participate largely in the formation and growth of the embryonic shield, but in somewhat later stages, this addition is found quite regularly, especially in the region of the posterior margin of the embryonic shield, which is furnished with a distinct primitive plate. The result of his observation, consequently, agrees fairly well with WILL's.

## XI. INTIMATE RELATION BETWEEN THE SUPERFICIAL ECTODERM AND THE SUBJACENT ENDODERMAL LAYERS

In the early stage, when the first indication of the invagination appears, GERHARDT observed fine threads communicating with the ectoderm and the endoderm, and, in the later stage, when the invagination cavity is completed, he found also a similar structure running from the endoderm to the primitive plate.

The result of the present writer's observation is, generally, allied to that, but his interpretation differs in detail from that, of previous investigators. In the present writer's material, also, in the position similar to that, observed by WILL, i. e. in the neighbouring portion of the embryonic shield, where the superficial ectodermal layer and the subjacent endodermal layer cohere with each other, each cell constituting the endoderm directs a protoplasmic process towards the ectoderm. Further, between the ectoderm and the endoderm, there is present a small number of cells, regarding which the present writer cannot decide whether they are ectodermal or endodermal, and, each of these cells is subject to the same process as that in the endodermal cell.

According to the facts given above, the inner layer may be considered as being a product by separation from the outer layer, and it may be presumed that the addition of a small number of cells from the superficial ectodermal layer to the inner endodermal layer occurs in this region.

In a stage with a more developed invagination cavity, the condition just mentioned is most remarkable in the region posterior to the embryonic shield, as BALLOWITZ states, and he shows a figure most applicable in support of the interpretation mentioned above.

As has been already stated, the present writer denies the occurrence of any addition of parablasic cells to the superficial layer in the material under investigation.

## XII. ORIGIN OF MESODERMAL CELLS

In regard to the origin of the mesodermal cells, different views have been previously put forward by several investigators.

KUPFFER reports having observed his 'Anhänge,' which are directed upwards and downwards connecting the 'Dotterblatt' (the primary endodermal layer of the present writer). The 'Anhänge' of KUPFFER corresponds presumably to the cell-aggregation in the present paper. He further maintains that the cells in the 'Anhänge,' which are stained



deeply as are the parablastic cells, migrate into the mesodermal region either isolated or in aggregated groups, participating largely in the formation of the mesoderm.

According to BALLOWITZ, in an early stage, the mesodermal cells originate from the cell-mass situated underneath the embryonic shield, on one hand, and the endodermal cells emanating from the cell-mass, on the other.

The present writer never observed such 'Anhänge' directed upwards from the endodermal layer as KUPFFER reports having found, and has no confidence in the truth of his statement. Further, the writer has ascertained that the peristomial mesoderm originates from the posteriorly and posterolaterally directed proliferation of the undifferentiated cell-mass forming the primitive plate in earlier stages, and that then there is a small amount of inward invasion of cells from the superficial layer on the posterior half of that plate, but that no addition of cells from the endoderm constituting a distinct single layer of spindle-shaped cells ever takes place. Even when the activity of the proliferation of the gastral mesoderm increases up to the stage immediately after the breaking through of the invagination cavity to the subgerminal cavity, the mesodermal cells and the endodermal layer are separated by a fairly distinct slit-like space, without there being any intimate connection between them.

On the degree of formation of the mesoderm, a remarkable difference is noticeable between the drawing made by KUPFFER (1882, fig. 17, *Coluber aesculapii*) and the result found by the present writer. The drawing by KUPFFER reveals that the mesoderm is insinuated far anteriorly beyond the head process into the space between the ectoderm and the endoderm, and forms a remarkable cell-sheet which is termed 'Axenplatte des Mesoderms' by him.

In the present writer's specimen, which corresponds to the stage of *Coluber* as drawn by KUPFFER, such a conspicuous insinuation of the mesodermal cells was never found, but only the ectodermal and the primary endodermal layers are visible there. In the case of *Elaphe quadrivirgata*, therefore, it is apparent that the formation of the mesodermal rudiment is noticeably more delayed than that in the case of *Coluber aesculapii*. Such an intimate relation between the mesodermal cells and the primary endoderm in this region, as shown in the drawing by KUPFFER, does not necessarily occur in the material under investigation.

As described above, the participation of the endodermal element in the formation of the mesoderm was never observed in any of the present

writer's specimens in which the mesoderm is in the course of being established.

### XIII. ORIGIN AND FORMATION OF THE CHORDA PRIMORDIUM AND THE INTIMATE RELATION BETWEEN THE CHORDA PRIMORDIUM AND THE PRIMARY ENDODERM

BALLOWITZ affirms that the cell-mass in the wall of the anterior extremity of the invagination cavity receives a quantity of cell-material from the threads of the parablastic cells in this region. KUPFFER obtains also the same result. The present writer, however, denies the occurrence of such a positive phenomenon.

In the material of the present writer, the turned-in anterior extremity of the wall of the invagination cavity arises in an intimate relation to the primary endoderm, which is already in position there, and these elements begin to combine gradually with each other with the progress of the gastrulation process. As WILL states, the combination becomes more complete, and finally both elements merge into each other, and it may be conceived that the addition of a small quantity of cell-material of the primary endoderm to the chorda primordium occurs in this region. The loosely associated cell-mass which consists of two elements, and which is described above, exists always in this portion. The portion is not settled and in a constant position, but changes anteriorly by degrees, owing to the forward transition of the anterior extremity of the invagination cavity, and, thus, the close relation between the primary endoderm and the chorda primordium is always continued in the anterior part of the latter.

Three factors are specified as contributing to the formation of the chorda primordium, viz. 1) thickening in the region of the dorsal wall of the turned-in endoderm (secondary endodermal element), 2) addition of a small amount of the primary endodermal element at the turned-in, anterior extremity, and 3) forward proliferation on the inner rim of the dorsal lip.

GERMARDT uses the term 'Mesodermsäckchen' for the walls of the invagination cavity, recognizing that the mesoderm is proliferated from the entire circumference of the cavity, and, therefore, his attention inclines towards the manner in which the chorda primordium originates from the mesoderm.

BALLOWITZ reports that the entire walls of the invagination cavity are formed by the transition of the cell-material in the primitive plate.

The present writer considers, as stated above, that the chorda primordium is originated by the endodermal descendants, and therefore regards

the term 'Axenplatte des Mesoderms' used by KUPFFER, etc. as inappropriate in this case. Consequently, he agrees with the interpretation put forward by WILL.

#### XIV. DIFFERENTIATION OF THE GASTRAL MESODERM

There is apparent evidence that the gastral mesoderm is originated by an active proliferation, directed somewhat antero-ventrally, from either lateral part of the dorsal blastoporic lip, immediately before the breaking through of the floor of the invagination cavity, and the formation of the mesoderm is delayed in time and so is later that of the chorda primordium.

#### XV. RELATION BETWEEN THE GASTRAL MESODERM AND THE CHORDA PRIMORDIUM

Resulting from such rapid proliferation, the anterior extremity of the gastral mesoderm, in a short time, attains almost the level of the anterior limit of the chorda primordium, and the anterior extremity of this tissue is placed far anteriorly to the level of the most anterior point in the median zone of the gastral mesoderm, which is far posterior to the anterior margin of either lateral wing which extends considerably by this time. Both the gastral mesoderm and the chorda primordium become continuous.

The gastral mesoderm has no connection with the primary endoderm, but its formation is, as stated above, exclusively participated in by the conspicuous proliferation from the superficial layer of either region lateral to the dorsal lip, and, as to the establishment of the gastral mesoderm, it is suggested also that the addition of a small amount of the invading cell-element, constituting the pre-existing chorda primordium on the lines of demarcation between them, takes place there.

#### XVI. POSITIONAL RELATION BETWEEN THE GASTRAL AND THE PERISTOMIAL MESODERMS

The stages of origination of both gastral and peristomial mesodermal elements are thus notably different, and the transverse line through the blastopore indicates approximately the original boundary between them.

#### XVII. MORPHOLOGICAL CHANGES IN THE ANTERIOR WALL OF INVAGINATION CAVITY, ESPECIALLY THE DEVELOPMENT OF THE HEAD PROCESS

While the invagination cavity deepens, there are found several notable morphological changes in its anterior wall.

WILL gives an illustration (1899, fig. 4) with a small head process, as a special case, owing to the concurrence of the formation of the invagination cavity and the head process. This drawing by WILL somewhat resembles figs. 5 and 6 drawn by GERHARDT and fig. 17 by KUPFFER; and fig. 3 by WILL which he notes as a normal case, nearly resembles text-figs. 9-12 drawn by BALLOWITZ, which show conditions of a conspicuous development of the head process. In general, the formation of the head process in *Elaphe quadrivirgata* is more delayed than that in *Tropidonotus natrix*. Consequently, it is usual for the process of invagination and the formation of the head process to concur. So, the present writer has never encountered such a successful formation of the head process, as mentioned above, and certifies that the results shown in fig. 4 by WILL, in figs. 5 and 6 by GERHARDT and in fig. 17 by KUPFFER are observed, even in the case of the present material, as a normal condition.

#### XVIII. RELATION OF THE PRIMARY ENDODERM TO THE ANTERIOR WALL OF THE INVAGINATION CAVITY

The primary endoderm creeps dorsally for a little while along the anterior face of the head process, and reaches a tolerable altitude. This condition resembles more or less that illustrated in fig. 4 by WILL and fig. 17 by KUPFFER, and shows an analogy with that of *Emys* by KUPFFER and of *Chelonia* by MITSUKURI.

#### XIX. INTUMESCENCE ON THE RIM OF THE DORSAL LIP

Immediately before the stadium of breaking through, when the total length of the invagination cavity scarcely reaches as far as the posterior one-third of the embryonic shield, a remarkable thickening occurs owing to the increase in the number of cells constituting the reflected inner wall on the rim of the dorsal lip in the present material. WILL observes first in *Tropidonotus natrix* such an appearance of the thickening after the completion of breaking through, and the invagination cavity extends considerably, reaching about as far as the posterior two-thirds of the embryonic shield. The specimens shown in fig. 5 by WILL and in VIII, in Pl. IX by the present writer belong to nearly the same stage, and, when compared with each other, a notable difference is seen. KUPFFER, BALLOWITZ and GERHARDT did not describe this distinct thickening in their materials.

## XX. FACTORS IN RELATION TO THE FORWARD DEEPENING OF THE INVAGINATION CAVITY

According to WILL, the posterior larger part of the invagination cavity is originated by the gradual deepening due to a simple invagination, which appears on the dorsal surface of the primitive plate, and its anterior part by the union of many irregular cavities which arise in the head process, and then this part communicates secondarily with the posterior one. In his fig. 5, the narrower portion, shown approximately in the middle of the entire cavity, is indicated as the point of union of both cavities mentioned above, and, thus, he has specified two causes of the formation of the invagination cavity.

In accordance with the result obtained by BALLOWITZ, the walls of the invagination cavity contain the cell-material of the primitive plate and show a marked tendency to the formation of the intercellular dehiscence, so that the deepening of the invagination cavity is not caused by a simple invagination of the ectodermal epithelium, but originates continuously from the dehiscences between cells.

The present writer observed the process of formation of the invagination cavity minutely in a complete series of different stages. Notwithstanding the previous statements, he obtained a negative result, i. e. the deepening of the invagination cavity is caused principally by a simple invagination of the ectodermal epithelium, and, then, a few small irregular cavities, which have arisen in the anterior and lateral walls are united secondarily to the pre-existing invagination cavity, when it has grown to its full extent.

In the present material, the whole length of the completed invagination cavity is, as a rule, very short, and as to its formation it is unquestionable that the intercellular cavities and dehiscences described by WILL and by BALLOWITZ are added to the formation of invagination cavity. In reality, the narrower part and the irregular unevenness of the walls shown in their drawings are not found in the material under investigation.

## XXI. LENGTH OF THE COMPLETE INVAGINATION CAVITY

The length of the completed invagination cavity reported by GERHARDT, BALLOWITZ and WILL is long, and differs much from that in the present material. The specimen shown in fig. 6 by WILL was in a stage just before the breaking through of the floor of the invagination cavity, and the length of its invagination cavity was about 1 mm., reaching nearly as

far as three-fifths of the total length of the embryonic shield.

The specimen shown in text-figs. 16 and 17 by BALLOWITZ was in a stage also immediately before the breaking through, and measured 0.36–0.45 mm. and in a subsequent stage 0.63–0.81 mm.

In the case of *Elaphe quadrivirgata*, the length of the invagination cavity is comparatively short, and, even when it attains its maximum length, measures about 0.7 mm., which is nearly constant in each individual and does not reach half the total length of the embryonic shield.

In the same stage, the breadth of the invagination cavity is widest at its anterior part, measuring 0.37 mm. and at its posterior part immediately inside the blastoporic opening, 0.26 mm.

#### XXII. BREAKING THROUGH OF THE INVAGINATION CAVITY TO THE SUBGERMINAL FLUID CAVITY, OR MORPHOLOGICAL CHANGES OF THE DEPRESSED FLOOR OF THE INVAGINATION CAVITY

In reference to the process of the disappearance of the cell-sheet between the invagination and subgerminal cavities, during reptilian gastrulation, various opinions have hitherto been put forward by several investigators.

WILL and GERHARDT observe that the area of breaking through is represented at first by a wall consisting of two layers, which came originally from different sources, and becomes enlarged by a gradual combination of small network perforations, caused by the numerous irregular interstices, which arise from intercellular dehiscences.

The opinion that is supported by WENCKEBACH represents that the area of breaking through is extended as a result of the lateral retirement of the wall in this portion.

The opinion of BALLOWITZ includes the two foregoing factors, and in substance is as follows:—

The floor of invagination cavity, consisting of loosely associated cells, becomes thinner, owing to its retreat, directed mainly forwards and slightly backwards. In the cell-material of this thin-walled area, numerous intercellular vacuoles arise, and some of them communicate with the invagination and subgerminal cavities. With the progress of the retreat of the floor, the perforation grows larger, and, finally, the breaking through of the whole area is complete.

The result obtained by the present observation is nearly allied to that of BALLOWITZ. The anterior extremity of the completed invagination cavity is directed somewhat dorsally, as mentioned before, and its anterior and immediately lateral walls consist of loosely associated cells forming meshes

of network. The region of the depressed floor, which is situated nearly in the centre of the structure, is observed as a double layer, for a while, consisting of cells of turned-in endoderm and of primary endoderm. The cells of the main floor of the invagination cavity are also associated somewhat loosely, having small irregular cavities between them, which is not so remarkable as what is observed in the anterior wall. With the progress of the process of breaking through, this region becomes so retracted, chiefly anteriorly and slightly laterally, that the floor becomes gradually thinner until the invagination cavity breaks through into the subgerminal cavity. As described above, the process of breaking through issues principally from the retraction, and the subsequent enlargement of the established passage is caused by the continuation of the retraction of the original floor and by the addition of the small cavities, which have been already formed in the anterior and immediately lateral walls.

#### XXIII. REMAINING ANTERIOR EXTREMITY OF THE INVAGINATION CAVITY AFTER THE BREAKING THROUGH

In the present material, the remaining anterior extremity of the invagination cavity after the breaking through, is no longer extended anteriorly or remaining for a long time as a pocket-like pouch, as ascertained to be the case in *Tropidonotus natrix* by previous investigators, and as soon as the breaking through is complete, it completely disappears. This fact shows a remarkable dissimilarity from the results obtained hitherto in the case of the Ophidia, and it is of interest that the condition in the present material rather resembles that in *Lacerta* as found by WENCKERACH, etc.

#### SUMMARY

1) The present investigation was undertaken in order to elucidate the process of gastrulation and the formation of the germinal layers in *Elaphe quadrivirgata quadrivirgata* (BORE).

2) When on the anterior portion of the primitive plate the first indication of the invagination appears, the primary endoderm has already formed, with a definite space between them, underneath the entire embryonic shield, and adheres to the underface of the annular zone and of the primitive plate.

3) The establishment of the primary endodermal layer is caused by an addition by the passing of parakeleptic cells or freely isolated cells from the yolk surface into the subgerminal fluid cavity, and on the circumference

of the blastoderm, the element of the primary endoderm continues to that of the germ wall. This condition is observable for a long time.

4) The factors in relation to the establishment of the primitive plate are as follows:—

i) The discontinuance of the columnar arrangement of the cells, which form the superficial epithelium on the posterior part of the embryonic shield.

ii) The considerable thickening caused by the active mitotic division of the ectodermal cells immigrated inside.

In this case, no addition of primary endodermal cells to the plate-substance was found, and the plate itself originates exclusively from the ectoderm.

5) In the case of the formation of the peristomial mesoderm, the following factors may be distinguished, namely:—

i) The insinuation of cells directed posteriorly and postero-laterally, which issue by division into the undifferentiated cell-mass of the primitive plate.

ii) The proliferation of the superficial ectodermal cells towards the inside in the posterior half of the primitive plate.

6) The primary endodermal cells have no relation to the formation of the peristomial mesoderm.

7) The deepening of the invagination cavity is caused mainly by a simple invagination, and slightly by the participation of several intercellular cavities in its turned-in anterior wall. These cavities are so slight in size as well as few in number, that they hardly enter into consideration in the case of the present material.

8) On the inner rim of the turned-in wall of the dorsal lip, there is found a remarkable thickening of cells, which projects towards the concavity formed on the corresponding wall of the ventral lip in the fully developed stage of the invagination cavity. The thickening disappears gradually until the time when the Kupfferian canal is completed. It is, therefore, a temporary structure.

9) Cells lateral and anterior to the tip of the invagination cavity are most loosely associated, forming a large number of variously sized, intercellular cavities and represent remarkable meshes of network. These cavities do not participate in the formation of the invagination cavity. In this region the primary and the secondary endoderms are completely combined with each other.

10) The breaking through of the invagination cavity into the sub-



germinal cavity is caused mainly by the retirement of tissue occurring in the region of the cell-sheet bordering both cavities. The addition of intercellular dehiscences participates slightly in the subsequent enlargement of the blastoporic passage.

11) The length of the completed invagination cavity is fairly short, and, therefore, the position of the anterior opening of the Kupfferian canal is situated comparatively posterior to the cavity.

12) With regard to the origin of the chorda primordium, the following factors are specified, namely:—

i) The thickening of the dorsal wall of the turned-in secondary endoderm.

ii) The invasion of some elements from the primary endoderm at the anterior part of the turned-in wall.

iii) The forward transition of cells on the turned-in rim of the dorsal lip.

13) In the present material the chorda primordium is, comparatively, poorly formed.

14) The gastral mesoderm arises chiefly from the prosperous inward proliferation of the superficial ectodermal cells at both sides of the blastoporic rim. It is suggested that some cell-elements of the pre-existing chorda primordium may be added to that of the gastral mesoderm at their boundaries.

15) The inner (anterior, ventral) opening of the Kupfferian canal becomes narrower than the outer (posterior, dorsal) one. It is suggested that the closure of this canal may occur, beginning at the inner part.

16) On the region slightly inside the original posterior lip of the blastopore, an inlet is observed directed caudally in the primitive plate.

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## EXPLANATION OF PLATE IX

I. Photomicrograph of one of longitudinal sections of specimen in Stadium I, the same as that in Fig. 3, showing contents of subgerminal fluid cavity in transitional region between embryonic shield and annular zone. Smaller parablasic cells are scattered in the comparatively upper part of subgerminal cavity in isolated and aggregated conditions. Larger yolk-laden parablasic cells, some of which are proliferating from yolk surface facing subgerminal cavity, are situated in a comparatively lower part. Moreover, a large yolk sphere is seen attached to yolk surface.  $\times 160$ .

II. Photomicrograph of one of the longitudinal sections through embryonic shield of specimen in Stadium II, same as that shown in Fig. 6, showing intimate relation between primary endoderm and aggregated parablasic cells. Ectoderm is not shown.  $\times 160$ .

III. Photomicrograph of median longitudinal section of specimen in Stadium I, same as that in Fig. 4, showing whole blastoderm and spacious subgerminal fluid cavity below. Primary endoderm is seen beneath ectoderm and clearly distinguished from ectoderm in region of embryonic shield, owing to presence of intervenient cavity between them.  $\times 80$ .

IV. Photomicrograph of median longitudinal section of specimen earlier in Stadium II, same as that in Fig. 5, showing well-developed thread-like aggregations of parablasic cells, attaching primary endoderm in region posterior to primitive plate. Intimate connection between ectoderm or cell-mass of primitive plate and primary endoderm is also clearly seen.  $\times 160$ .

V. Photomicrograph of median longitudinal section of specimen in Stadium II, showing intimate relation between ectoderm of annular zone and underlying primary endoderm. Each cell constituting primary endoderm projects protoplasmic process upwards towards ectoderm.  $\times 160$ .

VI. Photomicrograph of transverse section through embryonic shield of specimen, later in Stadium II, slightly later than that in Fig. 9 and same as that in IX in Pl. X, showing whole blastoderm and relation between ectoderm and primary endoderm.  $\times 80$ .

VII. Photomicrograph of longitudinal section of specimen in Stadium I, same as that in Fig. 3, showing transitional region between embryonic shield and annular zone. Multicellular ectoderm of embryonic shield passes by degrees to unicellular ectoderm of annular zone. Primary endoderm, which is spaced from ectoderm in region of embryonic shield, comes near that in annular zone.  $\times 160$ .

VIII. Photomicrograph of longitudinal sections of specimen in Stadium IV, slightly earlier than that in Fig. 15, in order of 1-5 from median to lateral, showing prominent intumescence at turned-in wall of anterior lip and floor of invagination cavity in course of breaking through in 1, laterally expanded anterior part of invagination cavity in 3, and primary endoderm, well-defined even in far lateral portion, to which lateral extension of chorda primordium does not reach, in 5.  $\times 80$ .

*a* anterior  
*am* anterior margin of embryonic shield  
*apc* aggregation of parablasic cells  
*az* annular zone of blastoderm  
*bt* breaking through of floor of invagination cavity  
*chp* chorda primordium  
*e* ectoderm of annular zone  
*gw* germ wall  
*ic* intervening cavity between ectoderm and endoderm  
*int* intumescence at turned-in wall of anterior lip  
*ipc* isolated parablasic cell  
*l* left

*lcp* lateral margin of chorda primordium  
*lm* lateral margin of embryonic shield  
*lpc* larger yolk-laden parablasic cell  
*mes* meshes of network  
*p* posterior  
*pe* primary endoderm  
*pm* peristomial mesoderm  
*prp* primitive plate  
*r* right  
*see* ectoderm of embryonic shield  
*sfc* subgerminal fluid cavity  
*spc* smaller parablasic cell  
*ssy* surface zone of yolk facing subgerminal fluid cavity  
*ys* yolk sphere

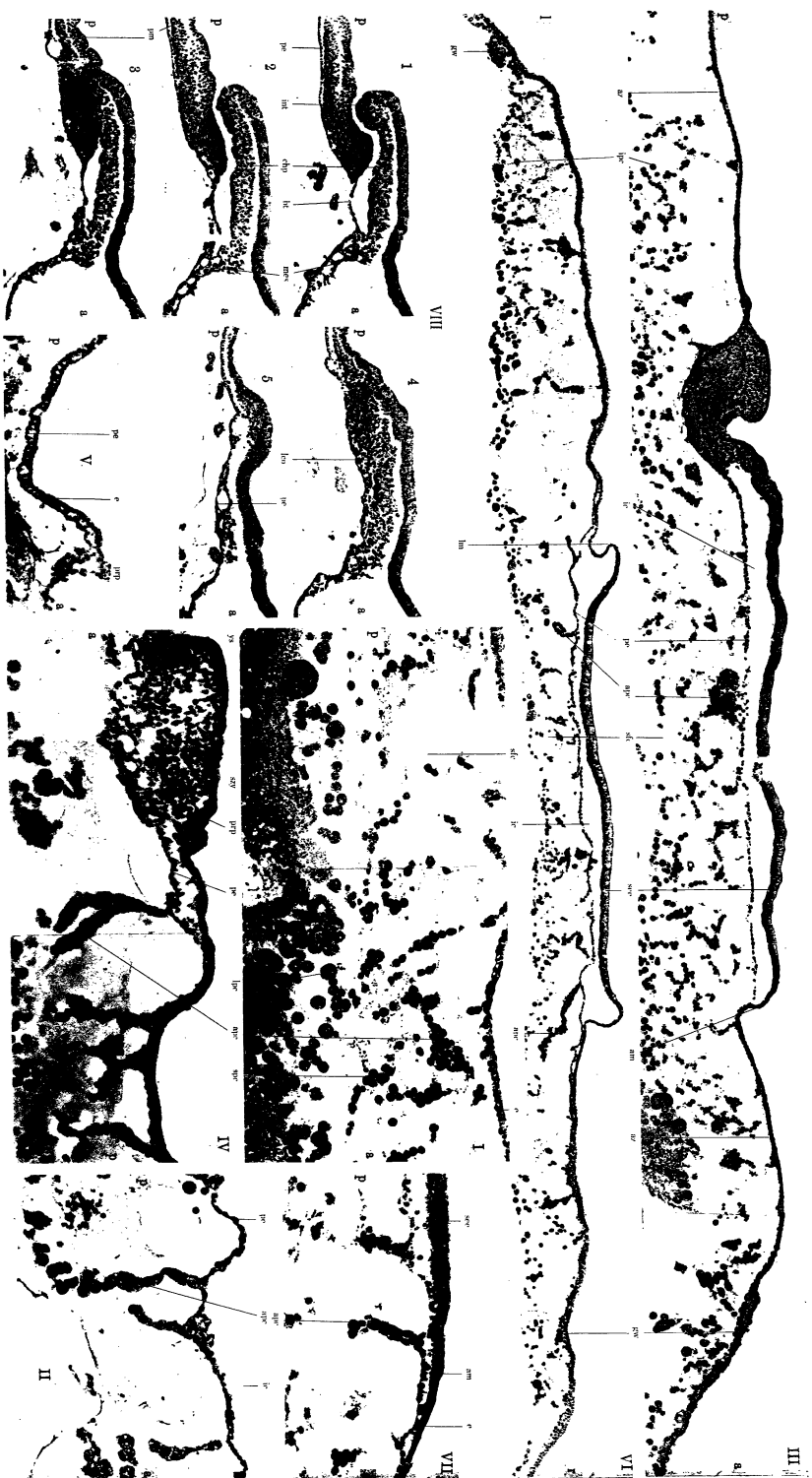
## EXPLANATION OF PLATE X

IX. Photomicrograph of transverse sections of specimen, later in Stadium II or slightly later than that shown in Fig. 8, in order of 1-13 from anterior to posterior, showing separation of anterior wall of invagination cavity from ectoderm in 2; combination of dorsal wall of invagination cavity with ectoderm in 4 and 5; posterior protrusion of anterior lip in 6; laterally extended blastoporic opening in 6 and 7; small knob of posterior lip in 8 and 9; ventral bulge of primitive plate towards subgerminal fluid cavity in 10; and loosely associated cells in cell mass of primitive plate in 12 and 13.  $\times 80$ .

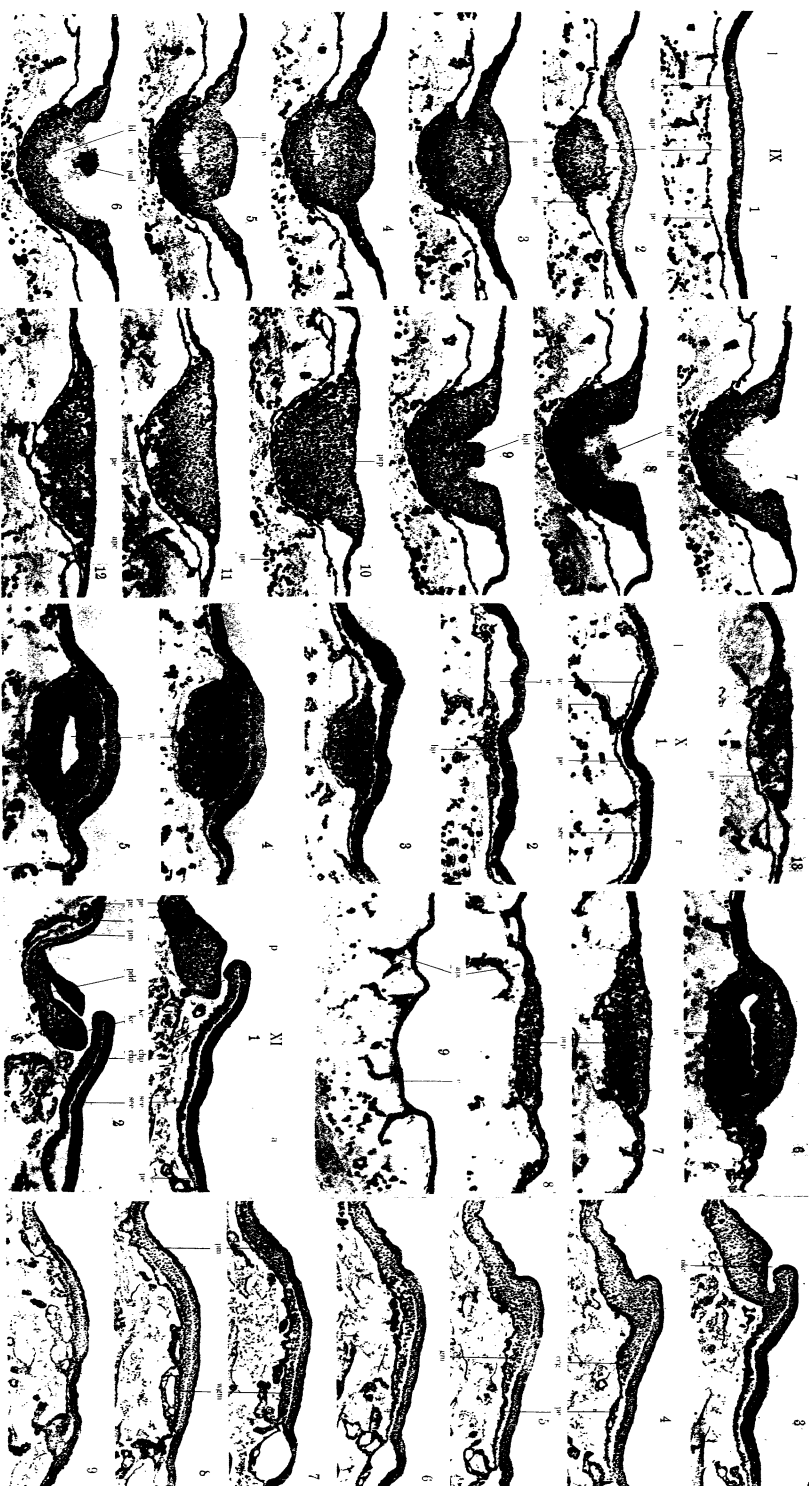
X. Photomicrograph of transverse sections of specimen in Stadium III, of nearly same age as that shown in Fig. 10, in order of 1-9 from anterior to posterior, showing anteriorly elongated, dorso-ventrally flattened end of head process in 2; most anterior end of invagination cavity in 4; elliptical outline of invagination cavity in its middle part, and equally thickened dorsal and ventral walls in 5; extremely dorso-ventrally flattened, but laterally extended invagination cavity at its posterior part in 6; and prominently formed aggregations of parablasic cells in region posterior to primitive plate in 9.  $\times 80$ .

XI. Photomicrograph of longitudinal sections of specimen in Stadium V, after completion of Kupfferian canal, in order of 1-9 from median to right, showing anteriorly much





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elongated chorda primordium, and similar thickness of posterior part of chorda primordium with overlying ectoderm in 1; posteriorly directed inlet in region of posterior lip in 2; gastral mesoderm as continuation of lateral border of chorda primordium in 4; peristomial mesoderm separated distinctly from overlying ectoderm and from underlying primary endoderm, which coheres intimately in anterior part of primitive plate, with its cell-mass, and gastral mesoderm proliferating actively from lateral part to Kupfferian canal in 4 and 5; antero-laterally extended gastral mesoderm forming wing, and continuation between gastral mesoderm and antero-lateral part of peristomial mesoderm at postero-lateral boundary of wing in 7 and 8; and intimate relation between primary endoderm and parablastic cells in 9.  $\times 80$ .

*a* anterior

*ap* anterior lip

*apc* aggregation of parablastic cells

*aw* anterior wall of invagination cavity

*bl* blastopore

*cag* cohesion between chorda primordium  
and gastral mesoderm

*chp* chorda primordium

*e* ectoderm of annular zone

*gm* gastral mesoderm

*hp* head process

*ic* intervenient cavity between ectoderm  
and endoderm

*ipc* isolated parablastic cell

*iv* invagination cavity

*kc* Kupfferian canal

*kpl* knob of posterior lip

*l* left

*okc* outer opening of Kupfferian canal

*p* posterior

*pal* posterior protrusion of anterior lip

*pdd* posteriorly directed inlet of invagina-  
tion cavity

*pe* primary endoderm

*pm* peristomial mesoderm

*prp* primitive plate

*r* right

*see* ectoderm of embryonic shield

*wgm* wing of gastral mesoderm





DOMINANCE AND AXIAL DIFFERENTIALS IN INDOPHENOL  
BLUE REACTION DURING RECONSTITUTION IN  
THE STALKED MEDUSA, *HALICLYSTUS*  
*AURICULA CLARK*<sup>1)</sup>

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(With twenty-seven figures)

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In the analysis of physiological factors that underlie control in development and reconstitution, it has become increasingly evident that orderly development and reconstitution depend upon a series of relations between dominant and subordinate regions of organisms. Various attempts to analyse this relationship of dominance and subordination has shown that dominance in the simpler organisms depends primarily on degree, not kind of physiological activity in protoplasmic substrata, and in consequence of the decrement in effect with increasing distance from the dominant region a "gradient" of physiological activity and protoplasmic condition results with the dominant region as its highest point or region. That is, the dominant region establishes a gradient which determines metabolic activities and morphogenetic processes at different levels in definite and orderly relation.

That physiological dominance is an essential factor in the organization of the individual organism is most conclusively shown in the inductive determination of individuals by experimentally localized dominant regions. In many of the lower organisms such dominant regions may be localized experimentally either by altering the physiological condition of a certain region or by grafting already determined dominant regions or parts capable of becoming dominant into new positions.

In an earlier paper on *Corymorpha palma* (WATANABE, 1935 b) it was shown that the polarity of an isolated piece of stem as indicated by reconstitution and by differential reduction of methylene blue is readily

<sup>1)</sup>Contribution from the Marine Biological Station, Asamushi, Aomori-ken, Japan, No. 141. An abstract of this paper was read at the twelfth annual meeting of Japanese Society of Zoölogists at Fukuoka, October 23rd, 1936.

altered or reversed, and is determined by the dominance of the original apical hydranth or developing hydranths. In these experiments the close parallelism between unipolarity or bipolarity in reconstitution and the reduction gradients constitutes evidence in support of the view that the reduction gradients are indicators of certain physiological characteristics of the stem axis. The polarity at the end of the piece is indicated by a new gradient, which is evidently an expression of the activation of the tissues following section and which precedes the appearance of the hydranth, i. e., from the higher levels of this gradient, the region of chief dominance develops.

It was thought desirable to investigate further the rôle of dominance in other coelenterates in maintaining or determining polarity, particularly since it seemed to show a relation to a differential in oxidative processes. The summer of 1935 and 1936 was spent at Asamushi Marine Biological Station in experiment along this line with a stalked medusa, *Haliclystus auricula* CLARK, which was found by CHILD (1933) to have rather remarkable capability for reconstitution. The results of the experiments are presented below. The writer desires to express his appreciation of the kindness of Professor S. HATAI LL.D., director of the Station, in providing space and facilities for the present work. Also to Dr. C. M. CHILD acknowledgement is due for suggestions and criticisms.

#### MATERIAL AND METHODS

**Material.** Stalk pieces 7–8 mm. in length from the largest individuals 23 to 25 mm. in length were employed as material for the present investigations, but the adhesive foot discs were discarded. The longer pieces are preferable for experiment, since during reconstitution, more or less decrease in size occurs in consequence of starvation and remodeling of old tissues.

For the purpose of distinguishing original distal and proximal ends, an oblique cut about 45° in angle was made at one and a transverse cut at the other end of the piece. With this procedure certain identification of the original polarity was possible. On the oblique surface of section the rate of reconstitution decreases in the proximal direction. This characteristic of development on an oblique surface is most clearly marked in the earlier stages of reconstitution, and becomes less marked as development proceeds, but is still evident 10 days after section. As CHILD (1933) has found, in the calyx of *Haliclystus* reconstitution at the proximal levels on an oblique cut surface is more or less inhibited, ap-

parently by the distal level. This relation in rate of reconstitution to the level of an oblique surface holds true also at the cut ends of stalk pieces (Figs. 11, 14, 15, 27). But the reconstitucional activity at a transverse cut of one end was found to be almost independent of, or at least little affected by, this inhibiting action from the distal level of an oblique surface at the other end of the piece. However, in order to avoid possible criticism on this point, in half the number of pieces in each experimental lot, the oblique cut was made at the distal end and the transverse cut at the proximal end, while in the other half, the oblique cut was proximal and the transverse cut distal (Figs. 7, 8, 18, 26).

In all cases animals were used for experiment within 2 days, after collection but were left at least one day in the laboratory without food.

**Reconstitution.** The method employed in the experiments on reconstitution was essentially that which was adopted in the writer's previous work on the *Corymorpha* stem (WATANABE, 1935 b). That is to say, control of polarity in the stalk by the original or a reconstituting calyx was examined by delaying either the distal or proximal section and by making a second distal or proximal section at different periods. The various operations are described in connection with particular experiments.

Each five operated pieces were kept together in filtered sea water in a bowl holding 250 cc., covered with a glass-plate to prevent evaporation. According to CHILD's findings (1933), death frequency in *Haliclystus* regenerates is generally lower when the water is not changed during a week or so, than with more frequent change. Consequently, half the volume of water in each bowl was changed every five days after operation. The pieces were moved about two or three times daily by means of water current from a pipett, and the bowls containing the pieces were submerged in running water in order to keep temperature as nearly as possible constant (18° to 20°C.).

Bipolar forms were separated as their character became evident, and 15 days after section, most forms were determined, but in a few cases regenerates were observed up to three weeks.

**Indophenol blue reaction.** As has been well known, this reaction consists in the production of indophenol blue by  $\alpha$ -naphthol and dimethyl-paraphenylenediamine in alkaline media. These reagents penetrate living cells and the intracellular reaction is catalyzed by so-called "nadi" oxidase. Differences in time of appearance and in amount of the colored particles of indophenol blue as estimated by color indicate the existence of physiological differentials of some sort in the cell or tissue concerned.

In applying this method to stalk pieces of *Haliclystus*, as to other forms, it is first necessary to determine, by trial, concentrations of reagents which give sufficient indophenol blue color in relatively short time to show differences in rate and intensity of reaction in different parts of the ectodermal layer of the stalk, but which are not appreciably injurious during the period of experimentation. In sufficiently low concentrations all parts of the ectoderm may show the reaction and certain parts may become deep blue while the pieces are still actively contracting, but in higher concentrations disintegration may occur before the reaction.

For the *Haliclystus* stalk, reagents were made as follows: 0.1 cc. of saturated dimethylparaphenylenediamine in sea water and 0.1 cc. of saturated  $\alpha$ -naphthol in sea water were mixed with 0.8 cc. of sea water in a large tissue-culture slide. The mixture was raised to pH 9.0 by addition of small quantity of sodium bicarbonate and a piece of stalk was immersed in the solution. With this procedure a characteristic indophenol blue color appears in the pieces, at first nowhere very deep, but with characteristic differentials in depth along the axis. As time proceeds, the color becomes deep and the differentials more clearly evident. In the earlier stages of reaction color differentials are much more clearly distinguishable, if the pieces are observed on a white background.

Since this reaction was employed primarily to show changes in condition of the stalk pieces in earlier stages of reconstitution, the reagents were applied 5 to 6 hours after final section, in order to avoid the temporary changes in condition following section. Immediately to 4 hours after section the indophenol blue always appears first and shows deepest color at the cut ends, and axial differentials of reaction shown by the pieces are irregular. Considered in the light of results of experiments described below (pp. 169-171), this coloration is regarded as indicating temporary disturbance of oxidase action or temporary change in physiological conditions caused by the stimulation of section and should be regarded as quite a different phenomenon from the oxidase activation intimately related to the reconstitucional activity at the cut ends.

*Figures and Graphs.* Figures representing reconstitution and indophenol blue reaction are semi-diagrammatic but from observed cases. Differentials in the indophenol blue reaction are indicated by depth of shading. The more deeply shaded parts indicate parts which are deep blue; the parts least shaded, parts in which diffuse blue is present in much lower concentration; and unshaded parts, regions in which color does not appear appreciably in the same period of exposure. These figures indicate only

the characteristic differences in reaction in different cases, but do not show the successive stages in the same pieces.

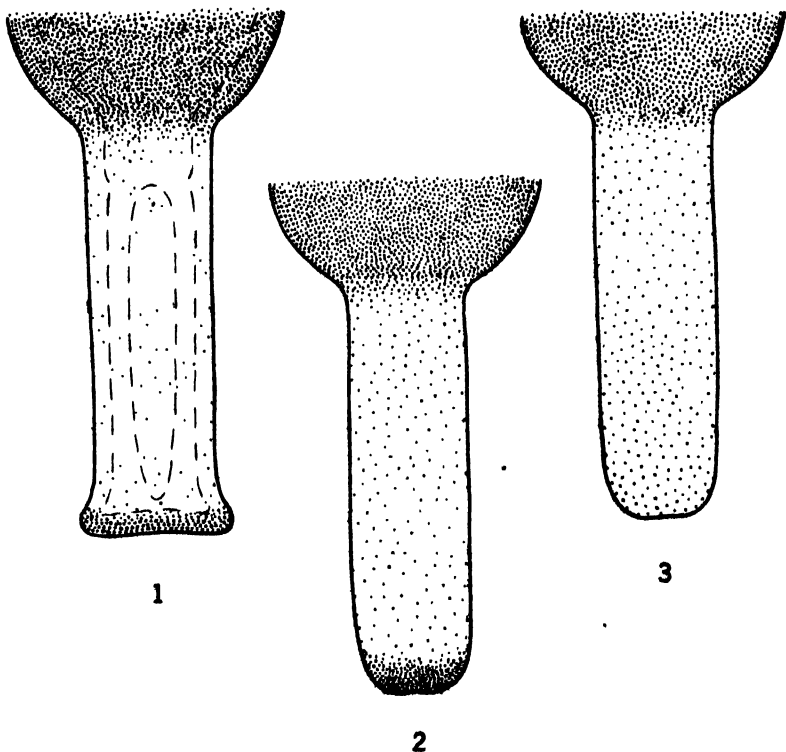
Most results of the experiments both on reconstitution and on indophenol blue reaction are graphed in percentages of unipolar forms as ordinates against periods of delay of distal or proximal section in hours as abscissae (Figs. 17, 21, 23, 25). Practically all cases not unipolar are bipolar, the frequency of other forms being so low that it is negligible. Death frequency in reconstitution was generally not over 5 per cent in each lot, with exception of one lot only in which 2 pieces out of 20 died. And frequency of apolar coloration in which no clear polarity in indophenol blue reaction could be observed, but blue color appeared uniformly along the whole length of pieces, was also not over 5 per cent in any lot. Totals were: in reconstitucional experiments 8 deaths in 400 pieces examined, i. e., 2.0 per cent; in the indophenol blue reaction 7 apolar in 441 pieces, i. e., only 1.7 per cent; all other pieces were either unipolar or bipolar forms. Therefore, the graphed data on unipolar forms may serve as indices of quantitative variations in dominance of original and developing calyces in relation to reconstitution and indophenol blue reaction.

#### DOMINANCE OF ORIGINAL CALYX AND POLARITY OF STALK PIECE

*Experiments on pieces with original calyx present.* As regards the differential indophenol blue reaction in intact animals, the writer's observation agrees well with the results obtained by CHILD (1933). The color of indophenol blue appears most rapidly in the anchors and tentacle tips, and next in the distal end of manubrium, then in the foot disc of unattached animals. The reaction progresses from distal to proximal on the tentacles, marginal lobes and manubrium, so that concerning the animal as a whole, a definite system of gradients in rate and intensity of reaction is observed. In the stalk region excepting the foot disc no definite differential was found, but reaction proceeds in general rather slowly and uniformly (Fig. 1).

As regards the reaction in operated pieces, first of all, the effect of stimulation from sectioning must be considered. The metabolic activities in the tissue concerned are abruptly increased and temporary disturbed by sectioning, as was already pointed out by the present writer (WATANABE, 1931, 1935 b; WATANABE and CHILD, 1933; CHILD and WATANABE, 1935). In the reaction immediately after section, coloration occurs most rapidly and most intensely at the cut surface, no matter whether the

original calyx is present or not (Fig. 2). This rapid coloration at the cut end immediately after section may indicate local activation of oxidase by stimulation from sectioning. But, as time goes on and stimulation decreases, depth and rate of coloration at the cut end also decrease, and about 5 hours after section, may be no greater than elsewhere in the stalk. Figure 3 shows the indophenol blue reaction 5 hours after proximal section in a stalk piece with original calyx present; coloration occurs



Figs. 1-3. Differentials in indophenol blue reaction in stalk and proximal part of calyx. Fig. 1, coloration in intact animal; Fig. 2, immediately after proximal section; Fig. 3, 5 hours after proximal section.

slowly and uniformly along the whole length of the stalk. From these results it is evident that in order to obtain a comparatively constant and approximately normal reaction in pieces, reagents should not be applied until at least 5 hours after section.

With this procedure no rapid coloration was found at the proximal cut ends of 20 pieces tested, from which the foot discs alone were removed.

ed, but the original basipetal polarity was indicated as in intact animals. In a reconstitution experiment on 20 stalk pieces with original calyces present, but not foot discs, new calyces did not develop at all from proximal cut end, the original polarity of the animals being also unchanged. The proximal cut surface healed (Fig. 3) and in some cases showed a slight development of foot-like structure 8 to 10 days after section (Fig. 4). However, the functional foot did not develop in any case for at least three weeks after section.

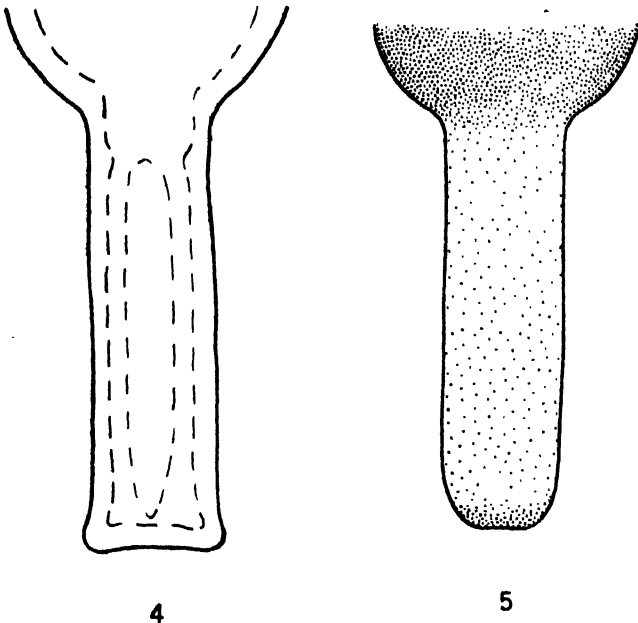


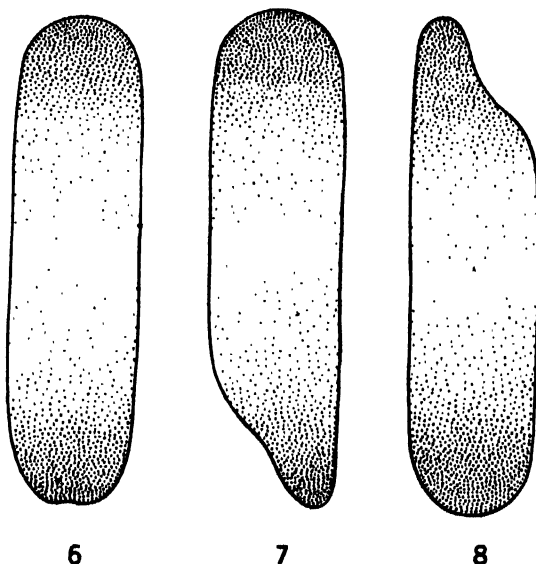
Fig. 4. Slight development of basal disc structure at proximal cut end of stalk, 9 days after section. Fig. 5. Uniform indophenol blue reaction in stalk region, with limited proximal reaction, 5 days after section.

In this connection, as an additional observation, the indophenol blue reaction was tried on the pieces 2 to 8 days after proximal section. The reaction at the proximal ends 2 to 4 days after section was still as slow as in the middle regions of the stalk (20 pieces examined), but 5 to 6 days or more after section in 10 pieces out of 20 a little more intense coloration appeared at the proximal ends. This coloration was, however, limited to a small area around the proximal end, and appeared a little more slowly than in the aboral umbrellar surface (80 to 100 minutes



after application of reagents), so that it is clearly distinguishable from the calyx gradient (Fig. 5). Considered in the light of the results with later stages, this region of somewhat intense reaction may perhaps be concerned later in development of a basal disc. Such a limited area of rapid reaction also appeared at the cut end in 5 days or more after section in a few pieces in other experiments on control by a developing calyx.

*Experiments on pieces without original calyx.* As expected from the results of the preceding experiments, indophenol blue color appears most rapidly and most intensely at the cut ends immediately after section, so that these pieces isolated by making distal and proximal section at the same time show bipolar coloration soon after cutting. Even more than 5 hours after section bipolar coloration was still observed in these pieces, but rate and intensity of coloration were more or less decreased, as compared with those observed soon after cutting. That is, blue color appeared at both ends of a fresh cut 10 to 20 minutes after application of the reagents, while it took 30 to 40 minutes to show appreciable coloration



Figs. 6-8. Bipolar gradient of indophenol blue reaction of stalk pieces with distal and proximal sections made at the same time, examined 5 hours after section. Fig. 6, piece with transverse sections at both ends; Fig. 7, piece with transverse distal and oblique proximal sections; Fig. 8, piece with oblique distal and transverse proximal sections.

there if the reagents were applied 5 to 6 hours after section (Figs. 6, 7, 8). This suggests that the activation of oxidase relating to the reconstitutive activity may begin at the cut end before the stimulation from sectioning disappears. In all 20 pieces examined 5 to 6 hours after section, coloration begins at both ends, progressing toward the middle at the same rate, and the last part to show the color is almost the middle, that is, they show typical bipolar coloration without exception (100 per cent bipolar).

These pieces both ends of which were cut at the same time almost invariably developed calyces at both ends. Out of 20 pieces examined, 19 pieces (95 per cent) reconstituted bipolar forms, and only 1 piece died during experiment. Figs. 6 to 15 indicate the various stages of reconstitution in these pieces up to 15 days after section. Immediately after section the cut ends of the pieces contract strongly, and within 4 to 5 hours after section the cut surface heals completely (Figs. 6, 7, 8). At 4 to 5 days both ends of the pieces usually begin to show some enlargement to form the calyces (Figs. 9, 10), and at the 6th to 8th day marginal anchors and single tentacles appear (Fig. 11). In shorter pieces, development of marginal organs sometimes occurs without distinct enlargement at both ends (Fig. 12). At 10 to 15 days pieces are distinct bipolar forms with calyces developed at both ends (Figs. 13, 14 and 15).

In pieces of *Corymorpha* stem, distal hydranth is always higher in rate and larger in scale of reconstitution than the proximal hydranth from the same piece. These characteristic differences between the two ends have been used in distinguishing the original polarity in the pieces of *Corymorpha* (WATANABE, 1935 b). In stalk pieces of *Haliclystus*, however, no constant difference between distal and proximal reconstituted calyces could be detected either in rate or in scale of reorganization. For this reason, an oblique cut was necessary at either distal or proximal end to indicate the original polarity.

For the indophenol blue reaction in pieces of later stages of reconstitution, 20 pieces of various stages were observed. They showed also distinct bipolar coloration, but coloration in the calyx regions became more rapid and more intense as their development proceeded. In these pieces, the last region to show color is also exactly the middle. Also young marginal organs and their buds showed most rapid and most intense coloration, as in the mature animals.

The parallelism shown by the data obtained above seems to suggest that there must be a certain essential relation between metabolic and

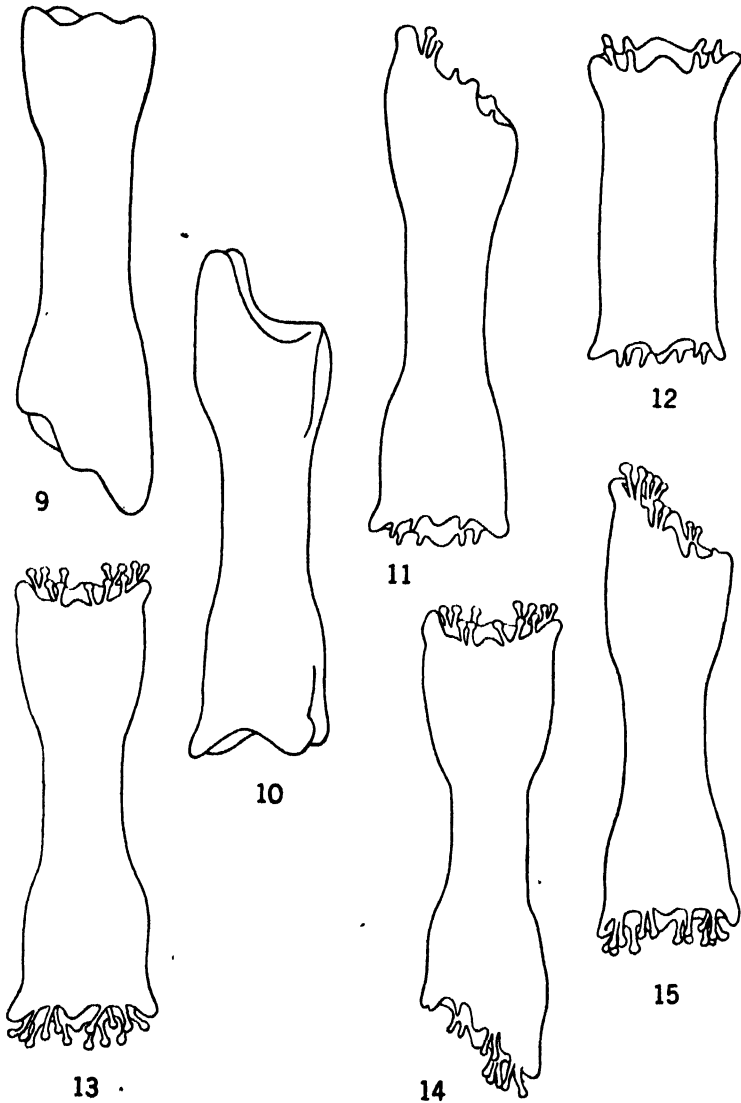
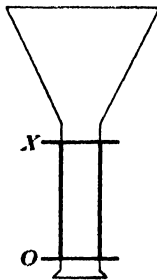


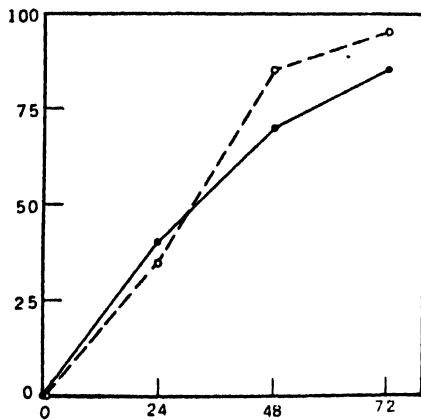
Fig. 9-15. Reconstitution in stalk pieces, with distal and proximal sections made at the same time. Figs. 9, 10, 5 days; Figs. 11, 12, 7 days; Figs. 13-15, 12 days after section. Figs. 9, 14, oblique cut made at proximal end; Figs. 10, 11, 15, oblique cut at distal end; and Figs. 12, 13, transverse cut at both ends. Fig. 12, reconstitution in short piece (ca. 1/2 stalk length), in which marginal organs develop without distinct enlargement of calyx.

morphogenetic activities at the proximal end, since both activities are controlled so exactly in the same way by the dominance of original calyx.

*Indophenol blue reaction and reconstitution with delayed removal of original calyx.* In the preceding experiments the pieces have been examined with respect to the effect of presence and absence of the original calyx, and it is clear that the original calyx exercises a dominance and through this dominance is able to prevent development of a new axis from the proximal end of the piece, both as regards indophenol blue reaction and in reconstitution. Since it may be expected that the inhibitory effect by the original calyx upon induction of a new polarity will increase with time after proximal section, the following experiments were performed to obtain experimental evidence and more definite and more nearly quantitative data concerning this apparent dominance. The proximal section was made (*O* in Fig. 16) and removal of the original calyx was delayed 0, 24, 48 and 72 hours (*X*, Fig. 16), 20 pieces being used for each period of delay.



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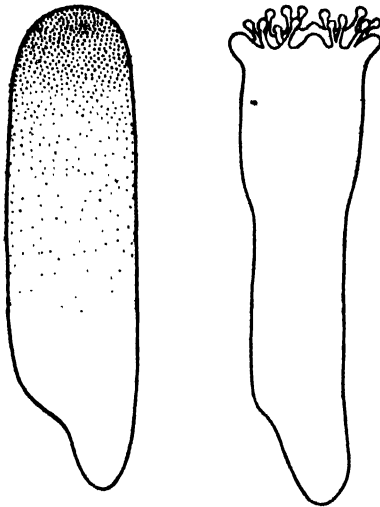


17

Figs. 16, 17. Fig. 16, experimental procedure; proximal section at *O* hours, distal section at *X* (0, 24, 48, 72) hours. Fig. 17, graph of results; ordinates, percentages of unipolar frequency, abscissae, hours; unbroken line, indophenol blue reaction; broken line, reconstitution.

The pieces with delayed removal of the original calyx, which were exposed to nadi reagents 5 hours after final section, show either unipolar or bipolar reaction, but never unipolar reaction without rapid coloration in the distal region, that is, in no case does a complete reversal of polarity in the reaction occur. The percentage frequencies (ordinates) of hasipetal-unipolar reaction (Fig. 18) are graphed in unbroken line in Figure 17 for

the various periods of delay (abscissae). As the graphed data show, of 20 pieces, with 0 hour delay of calyx removal, none shows unipolar reaction; of the second 20 with 24 hour delay, 8 pieces (40 per cent) are unipolar; of the third 20 with 48 hour delay, 14 pieces (70 per cent) are unipolar; and of the fourth 20 with 72 hour delay, 17 pieces (85 per cent) are unipolar in reaction.



18

19

Figs. 18, 19. Basipetal unipolar pieces, obtained by 72 hours delay of distal section. Fig. 18, complete basipetal differential in indophenol blue reaction, 6 hours after final section. Fig. 19, reconstitution of piece, 14 days after the first section.

The results of reconstitution experiments on the pieces with delayed removal of the original calyx are given in broken line in Figure 17. In reconstitution also, pieces developed either basipetal-unipolar forms or bipolar forms, but never acropetal forms. The graphed data show that the percentage of unipolar forms (Fig. 19) also increases with increase in period of delay of calyx removal. With 0 hour delay the graph shows 0 per cent (0 pieces); with 24 hour delay, 35 per cent (7 pieces); with 48 hour delay, 85 per cent (17 pieces); and with 72 hour delay, 95 per cent (19 pieces).

By reference to these graphs, it will be seen that the frequency of unipolar cases increases almost uniformly from 0 to a very high per cent (85 or 95 per cent) with delay of calyx-removal from 0 to 72 hours. In other words, the longer the original calyx remains the more frequently is formation of a calyx as well as activation of nadi oxidase in the proximal region inhibited.

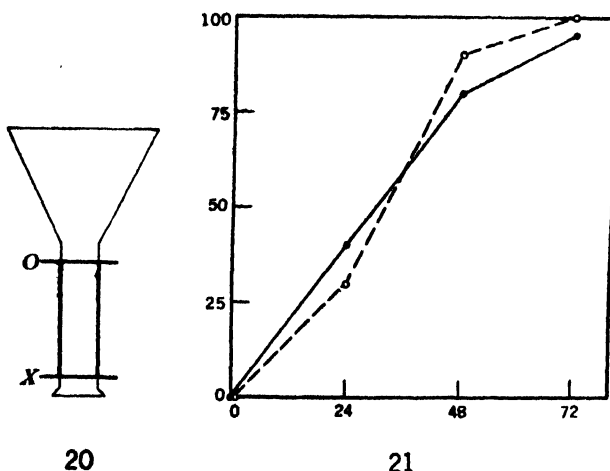
#### DOMINANCE OF DEVELOPING CALYX

As the preceding data show, almost all stalk pieces develop calyces at both ends and show distinct bipolar coloration of indophenol blue when both sections are made at the same time, while no cases of biaxial calyces or of biaxial nadi reaction appear, if fully developed calyces are present. Consequently it may be expected that a calyx developing at one end of

the piece will become a dominant region and inhibit the development of another dominance at the other end. In order to test these possibilities, the following experiments were undertaken.

*Indophenol blue reaction and reconstitution with delay of proximal section.* In this series, the proximal section was delayed 0, 24, 48, 72 hours after the removal of the original calyx (Fig. 20), 20 pieces being used with each period of delay for the indophenol blue reaction and for reconstitution, respectively. In these experiments the distal calyx is of course 0, 24, 48 and 72 hours more advanced in reconstititional development than the proximal.

The results are graphed in Figure 21, with percentages of unipolarity in indophenol blue reaction (unbroken line) and in reconstitution (broken line) as ordinates and delay of proximal section in hours as abscissae.



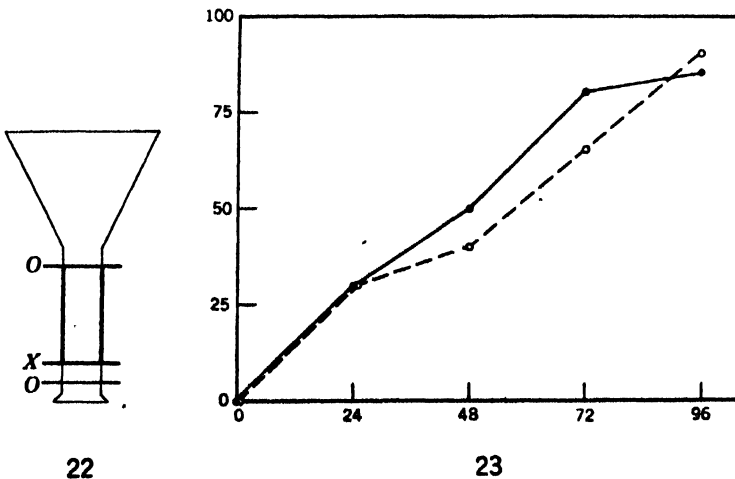
Figs. 20, 21. Indophenol blue reaction and reconstitution with delay of proximal section. Fig. 20, experimental procedure; distal section at *O* hours, proximal section at *X* (0, 24, 48, 72) hours. Fig. 21, graph of results; ordinates percentages of unipolar frequency, abscissae, hours; unbroken line, indophenol blue reaction; broken line, reconstitution.

Needless to say, in these unipolar pieces the coloration appears always basipetally or the calyx is always at the distal end. Percentage frequency of unipolar indophenol blue coloration (cf. Fig. 18) increases from 0 to 40 per cent with 24 hour, 80 per cent with 48 hour, and 95 per cent with 72 hour delay of proximal section. And percentage frequency of

unipolar reconstitution (cf. Fig. 19) also increases with increasing period of delay of proximal section: 0 per cent in the first lot, 30 per cent with 24 hour, 90 per cent with 48 hour and 100 per cent with 72 hour delay.

As these data show, frequencies of unipolarity in indophenol blue reaction and in reconstitution seem to be practically the same with the same period of delay of section, and they increase almost proportionally to the period of delay. And finally with sufficient delay of the proximal section the developing distal calyx does completely inhibit development of a proximal dominance as the original apical calyx does. In other words the effectiveness of the distal calyx as a dominant region increases as its development progresses.

*Experiments with delayed second proximal section.* This series is supplementary to the preceding. As shown above, in the course of reconstitution, action of a developing distal calyx upon the development of a proximal dominance with establishment of reversed polarity increases as its development progresses. If this is due to development of dominance in the new calyx at the distal end of the piece, it should be possible to increase unipolar frequency by a delayed second proximal section in pieces in which the first distal and proximal sections had been made at the same



Figs. 22, 23. Indophenol blue reaction and reconstitution after a second proximal section at different periods of delay. Fig. 22, experimental procedure; distal and first proximal sections, O hours, second proximal section at X (0, 24, 48, 72, 96) hours. Fig. 23, graph of results; ordinates, percentages of unipolar frequency, abscissae, hours; unbroken line, indophenol blue reaction; broken line, reconstitution.

time. In order to examine this possibility the following additional experiments were performed.

The original calyx was removed and the first proximal section was made at the same time (distal and proximal *O*, Fig. 22) and a second proximal section was made at *X*, ca. 2 mm. from the proximal end at 0, 24, 48, and 96 hours after the first sections, lots of 20 pieces for each period being used for indophenol blue reaction and for reconstitution.

Percentage frequencies of basipetal-unipolar pieces resulting, which are graphed in Figure 23, show continuous increase, in indophenol blue reaction (unbroken line) from 0 per cent in the first lot to 30 per cent with 24 hour, 50 per cent with 48 hour, 80 per cent with 72 hour and 85 per cent with 96 hour delay, and in reconstitution (broken line) also from 0 per cent in the first lot to 30 per cent with 24 hour, 40 per cent with 48 hour, 65 per cent with 72 hour and 90 per cent with 96 hour delay of second proximal section. Evidently, as in preceding experiments, the dominance of the distal calyx increases as reconstitution proceeds.

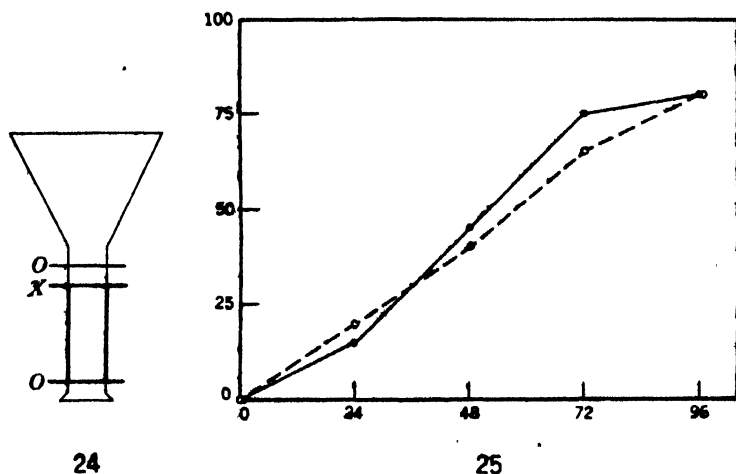
Comparison of these data (Fig. 23) with those in the preceding experiments outlined in Figure 20 and graphed in Figure 21 shows that in the present series the increase in unipolar frequency is much less than that with corresponding periods of delay of section in the preceding, excepting frequency of indophenol blue reaction with 24 hour delay. And both in indophenol blue reaction and in reconstitution, its increase even at 96 hours (85 per cent and 90 per cent) in the former is still less than that at 72 hours (95 per cent and 100 per cent) in the latter. That is to say, in general, with delay of second proximal section, many more pieces develop proximal dominance and, in consequence, bipolar forms than with delay of single proximal section. As shown above (Figs. 6-8), in pieces with distal and proximal sections made at the same time, the indophenol blue coloration is bipolar and its proximal gradient extends from the proximal end to the middle of the pieces. This suggests that, before a second proximal section is given, physiological activity has been increased to some extent at *X* (Fig. 22) by the development of dominance at the proximal end, *O*. Accordingly, it should be expected that the calyx primordium at the distal end, *O*, will be less effective in inhibiting calyx development as well as the development of an active pace-maker as indicated by the indophenol blue reaction at the proximal end than if such activation had not occurred. Figures show that this is the case.

*Experiments with delay of second distal section.* In the two preceding series, the dominance concerned is the dominance developing at the distal



end of piece, and the increase in frequency of basipetal unipolarity is determined. In the present series the attempt is made to determine the effect at the proximal end and the frequency of acropetal unipolarity. The distal and proximal sections were made at the same time (O, O in Fig. 24) and a second distal section was made at X, ca. 2 mm. from the distal end of the piece at 0, 24, 48, 72 and 96 hours later with 20 pieces in each lot for indophenol blue reaction and for reconstitution. The percentage frequencies of unipolarity are given in Figure 25.

The pieces thus operated become either bipolar or acropetal-unipolar (Fig. 26, 27), but never basipetal-unipolar, that is, no cases of indophenol blue coloration progressing proximally throughout from the distal end, nor of development of a distal calyx without development of a proximal calyx

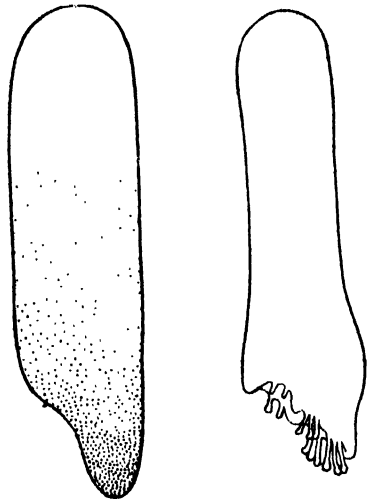


Figs. 24, 25. Indophenol blue reaction and reconstitution after delayed second distal section at different periods. Fig. 24, experimental procedure; first distal and proximal section at O hours; second distal section at X (0, 24, 48, 72, 96) hours. Fig. 25, graph of results, ordinates, percentages of frequency of acropetal unipolarity; abscissae, hours; unbroken line, indophenol blue reaction; broken line, reconstitution.

occur. As the graphed data show, frequency of acropetal unipolarity increases almost uniformly from 0 to 80 per cent with increase in delay of second distal section up to 96 hours, both in indophenol blue reaction (unbroken line) and in reconstitution (broken line). Consequently, it is evident that the dominance of the developing calyx at the proximal end as shown by inhibition of dominance and development at the distal end, X, increases as reconstitution progresses.

Strictly speaking, in Figure 25 the increase of unipolar frequency is, in general, slightly less than in Figure 23, for delay of second proximal section, but the difference is so small that it is permissible to conclude that the effects of delay of second distal section and of second proximal section on unipolar frequency are nearly the same. Comparison of the graphs, Figures 23 and 25, shows clearly that the increase of dominance of distal and proximal calyx primordia occurs approximately at the same rate.

The development of distal and proximal dominance at the same rate is undoubtedly correlated with the results obtained with pieces sectioned at both ends at the same time. As shown above (pp. 172-174 and Figs. 6-15), rate and scale of reconstitution of calyx are almost the same at both ends in these pieces, and distal and proximal gradients of indophenol blue reaction are also equal in length, each extending over half the length of pieces from each end. In other words, there is little evidence of polarity in the stalk or of any effect of such polarity, if present, on rate of indophenol blue reaction or on scale of reconstitution at the two cut ends of stalk-pieces.



26

27

Figs. 26, 27. Completely reversed pieces of stalk after delayed second distal section. Fig. 26, acropetal coloration of indophenol blue; Fig. 27, reversed form reconstituted with 96 hour delay of second distal section.

#### DISCUSSION

Evidence that the original hydranth and the developing hydranth primordium of a gymnoblastic hydroid, *Corymorpha palma*, exercise a certain degree of dominance or control of the physiological polarity of the stem pieces has been given by the writer in studies on reconstitution and methylene blue reduction (WATANABE, 1935 b). As a further contribution to the subject, the data, presented in this paper extend the analysis of dominance to a sessile medusoid scyphozoan, *Haliclystus auricula*, and they bring to light certain facts of importance concerning physiological

dominance of the calyx in this form. Attention may be called to the fact that the calyx of *Haliclystus* corresponds in position to the hydranth of *Corymorpha* and is undoubtedly homologous to it. The experiments show first that the presence of a fully developed calyx inhibits calyx development at the basal end of the stalk; second, that a new calyx primordium developing after section is increasingly effective up to a certain stage of development in inhibiting calyx development at the opposite end of the stalk piece; and third, that these dominant regions, both original and newly established, determine the range and direction of control as indicated by the differential oxidative reaction of the nadi enzyme, the highest end of the gradient thus established being always the calyx region.

In intact animals the stalk region except the adhesive foot disc does not show any distinct differential in rate of indophenol blue reaction. As compared with other regions of the body, reaction occurs there most slowly and quite uniformly. In the stem of the mature *Corymorpha*, reduction of methylene blue (CHILD and WATANABE, 1935) and the nitroprusside color reaction (CHILD and HYMAN, 1926) become evident first at the distal part and progress proximally over the naked region almost to the perisarc. In younger forms, 15–20 mm. in length or longer, the indophenol blue reaction also shows a distinct main basipetal gradient with a short perisarc gradient in the opposite direction (CHILD, 1926). These data indicate that the *Haliclystus* stalk differs from the *Corymorpha* stem in possessing little or no axial differential, consequently an analysis and comparison of reconstitution at both ends of pieces of these two forms are of interest.

As already noted, the piece with distal and proximal sections made at the same time invariably develops a calyx at both ends, and distal and proximal calyces are almost equal in scale of reorganization as well as in rate of development. According to CHILD (1933), no difference in rate of reconstitution was observed in the *Haliclystus* stalk after transverse section at the distal end and the middle. These characteristics of calyx development in *Haliclystus* are quite different from hydranth reconstitution in *Corymorpha* and also in *Tubularia*. In pieces of these forms which are not extremely short and which are equally exposed at both cut ends, the distal hydranth develops in general somewhat earlier than the proximal hydranth, and its scale of organization is usually larger than that of the proximal (for example, CHILD, 1907, 1926; CHILD and HYMAN, 1926; DRIESCH, 1899; HYMAN, 1920; MORGAN, 1906, 1908; MORGAN and STEVENS, 1904; WATANABE, 1935 b). In other words, in the *Haliclystus* stalk,

the physiological conditions chiefly concerned in calyx formation appear to be approximately the same at both ends, while in the hydroid stem the original physiological gradient determines a difference. That is to say, although the two cut ends are nearly equipotential in the hydroid stem in respect to the capacity for hydranth development, the influence of the original gradient is still evident in the difference in rate and scale of hydranth reorganization. In a recent work on another stalked medusa, *Thaumatoscyphus distinctus*, HANAOKA (1935) has found an axial differential in the stalk region in the potency for reconstitution of tentacles and in the histological character of tentacles reconstituted. According to these data the stalk of *Thaumatoscyphus* is somewhat different from the present material in axial constitution, and is more like the hydroid stem. However, as other lines of evidence for *Thaumatoscyphus* have not been given, discussion of this apparent difference between these two species remains for the future, though the difference seems to be of interest. At any rate, "restitutional development is of particular interest for the study of physiological gradient", as the writer has suggested, "since it enables us to learn something about the part which the original gradient plays in determining the new pattern and the changes which it undergoes as the new pattern is determined" (WATANABE, 1935 a).

The influence of the original gradient of intact animals as shown in restitutional development can also be clearly seen in oxidative and reductive processes in isolated pieces, if examination is undertaken after the cut end has closed and stimulation by sectioning has disappeared. In the bipolar gradient of methylene blue reduction in pieces of *Corymorpha* stem with distal and proximal sections made at the same time, the distal gradient usually appears a little earlier and is a little longer than the proximal gradient; consequently, the last part of the pieces to reduce is somewhat proximal to the middle (CHILD and WATANABE, 1935; WATANABE, 1935 b). As regards the length of gradients, the bipolar gradient of indophenol blue reaction in stalk pieces of *Haliclystus* with distal and proximal section at the same time differs from that of *Corymorpha*. In these pieces of *Haliclystus* stalk, the indophenol blue reaction, like calyx development, occurs at the same time at both ends and progresses at the same rate, both distally and proximally, so that the last part to show the color of indophenol blue is almost invariably the middle of the pieces (Fig. 6, 7, 8). This bipolar reaction with distal and proximal gradients of equal length persists during calyx reconstitution except as it is regionally changed by differentiation of marginal anchors, tentacles and manu-

brium. The difference in relative length of distal and proximal gradients resulting from section in these two forms is evidently due to presence of a gradient in the intact *Corymorpha* stem and absence in the *Haliclystus* stalk.

However, as the results of tentative experiments show, even in these pieces this bipolar gradient does not always appear immediately after section and the reaction shows considerable variations. In some cases no axial differential appears; in others, a basipetal gradient; in still others, a bipolar gradient; and in a few, an acropetal gradient. But, as time proceeds and effect of stimulation by sectioning decreases, the definite bipolar coloration appears. Consequently, these variations should be regarded as temporary disturbance caused by section and isolation. This temporary disturbance following section is also shown by pieces with original calyx present: immediately after proximal section the intense coloration at the cut end and, occasionally, a proximal acropetal gradient appear, while 5 to 6 hours after section they are no longer present, but the reaction occurs quite uniformly over the whole length of the stalk region (Figs. 2, 3).

As regards these wide variations following section, HYMAN (1923) has shown that the rate of respiration in *Planaria* is temporarily increased by section, and according to her observations on *Nereis* (HYMAN, 1932), differences in rate of oxygen uptake based on the data obtained during the first 3 hours after separation into pieces are different from those based on the data during the second 3 hours, the latter showing a distinct double gradient. The writer's own observations on CO<sub>2</sub> output in earthworms, *Allolobophora foetida* (WATANABE, 1931) and *Pheretima hilgendorfi* (WATANABE, unpublished data), and in a polyclad, *Stylochus ijimai* (WATANABE and CHILD, 1933) also show that very wide variations in rate of pieces from different regions of body occur following section, and that a definite form of axial gradient does not appear at once, but, as the effect of section decreases, a distinct U-shaped gradient becomes evident two to three hours or more after section. In recent work on *Corymorpha palma*, the variations in methylene blue reduction following section also indicate the temporary disturbance of physiological condition resulting from sectioning. Various factors may be concerned in these variations. In the first place, a temporary excitation undoubtedly occurs, particularly at and near the cut ends, and apparently undergoes rather rapid decrease, sometimes different rate in the two ends of pieces, perhaps, because of differences in cell injury. It may be suggested that, if it were possible to bring

about dye reduction or indophenol blue reaction in a few seconds after section, we might discover definite and characteristic conditions immediately after section although they might be different in some way from the later stages. At present, however, it is impossible to obtain any evidence on this point because available methods are inadequate for the purpose.

Returning to the data of the present paper, the pieces with the original calyx do not develop a proximal calyx. Unquestionably this indicates the dominance of the fully developed calyx in maintaining the original polarity of animal. In these pieces indophenol blue reaction 5 to 6 hours or more after proximal section does not show an axial gradient from the proximal end. But 5 to 6 days after section, an increased reaction begins to appear at the proximal cut end of the stalk, in spite of the presence to the original calyx. The proximal reaction, however, is limited to the extreme proximal end and appears a little later than the reaction in any part of the calyx region (Fig. 5). As suggested by CHILD and WATANABE (1935) and WATANABE (1935 b) with respect to a proximal region of more rapid reduction in unipolar *Corymorpha* pieces, this limited coloration at the proximal end may represent the beginning of development of a basal structure. If this is the case in the *Haliclystus* stalk, this increased activity at the proximal end precedes 3 to 4 days the development of visible structure, since a slight development of a foot-disc occurs 8 to 10 days after section (Fig. 4). In these pieces the indophenol blue reaction does not show equal bipolar gradients at any time, but only a unipolar-basipetal gradient in earlier stages and a second short basal gradient in later stages of reconstitution.

If the original calyces are removed at different times after proximal section, the frequency of bipolar gradients and bipolar forms is greatly increased (Fig. 17). It is suggested that the dominance of the original calyx prevents or partially prevents the activation of cells and the development of a new gradient proximally, which appear to be the most essential factors in the reconstitution of a calyx at the proximal end of the stalk, and that dominance of the distal cut region after removal of the original calyx is not effective to an appreciable degree. When the original calyx is present, the cells at and near the proximal cut are subjected to correlative conditions which tend to maintain their characteristics as stalk cells or to form them into a basal disc, and if these factors are sufficiently effective, the cells are prevented, even after distal section, from undergoing the physiological activation necessary for establishment of a new gradient distally from the proximal end and attainment of the threshold

of calyx development.

When the original calyx is removed and proximal section is delayed (Fig. 20), development of a proximal gradient and proximal calyx is also greatly inhibited, so that frequency of bipolar gradients and bipolar forms is decreased. With sufficient delay (72 hours or more) of proximal section the developing calyx primordium at the distal end becomes dominant and prevents development of calyx and calyx gradient from proximal end (almost 100 per cent basipetal-unipolar, Fig. 21). When distal and proximal sections are made at the same time and the distal calyx primordium is removed by a delayed section at different times after the first (Fig. 24), the developing calyx at the proximal end becomes dominant. As the period of delay of the second distal section increases, frequency of bipolar gradient and calyx development at distal end decreases. With sufficient delay (96 hours or more) of the second distal section, the proximal calyx primordium prevents the development of indophenol blue gradient and calyx from the second distal end in very high percentages (Fig. 25). Needless to say, in such cases the reconstituted animals are unipolar but the axial order is completely reversed in direction (Figs. 26, 27).

The dominance of the calyx primordium, at either distal or proximal end, increases as development progresses; and when sufficiently effective, it prevents development of a new gradient and a new calyx at the opposite end of the piece. That is to say, in earlier stages of calyx development, the range of dominance may be very short, probably covering only the limited area close to the cut end immediately after section, but as calyx development progresses the range of dominance may increase. And finally when it extends over the whole length of the piece, the other end is not physiologically isolated, and establishment of a new gradient is inhibited. Consequently, it is possible by sufficient delay of the second distal section to reverse completely the axial order.

As the data obtained from the experiments with delayed second distal section and with delayed second proximal section (Figs. 22, 24) show, the development of dominance of distal and proximal primordia occurs almost at the same rate. This affords further evidence of equipotentiality of calyx formation at both ends of stalk region of *Halicylistus*. Comparison of these data (Figs. 23, 25) with those from the experiments with single delayed proximal section (Fig. 21) shows, however, that the increase of unipolar frequency is much less rapid in the former cases than in the latter. As the indophenol blue reaction shows, the gradients in bipolar forms are established from both ends toward the middle of the piece

(Figs. 6-8). This evidence from the indophenol blue reaction indicates that a gradient at *X* (Figs. 22, 24) has been determined by the development of calyx primordium at the end of the piece, *O*, close to it. If this is the case, it is to be expected that a calyx primordium at a distal (or proximal) end will be less effective in inhibiting establishment of reaction gradient and development of calyx from the level of the second proximal (or distal) section, *X*, than if a gradient and dominance had not already been established as a result of the first proximal (or distal) section at *O*.

The results of the experiments of the present paper are in agreement with the writer's previous work on *Corymorpha* pieces (WATANABE, 1935 b) in showing close parallelism between unipolar frequency of a reaction related to physiological oxidation and reconstitution. The new polarity of the stalk piece of *Haliclystus* is indicated by a gradient of indophenol blue reaction which is unquestionably based upon the activation of nadi enzyme in the cells following section, and from the higher level of the gradient, the chief dominant region develops. This gradient of indophenol blue reaction becomes visible 3 to 4 days before the form of the calyx is distinguishable. This general correspondence between gradient of physiological oxidation at an earlier stage and determination of morphological polarity which becomes evident at a later stage of reconstitution is rather striking, and leaves little doubt that such physiological differentials are an essential factor in determining calyx development and new polarity. In conclusion, it may be emphasized that physiological dominance determines a gradient of certain extent which in turn determines morphogenetic processes as well as physiological activities of subordinate regions in definite and orderly sequence.

#### SUMMARY

1. The paper presents data on experimental control of physiological polarity in stalk pieces of *Haliclystus auricula* as shown by differentials in indophenol blue reaction and in reconstitution of calyx.
2. The stalk region of intact animals does not show any definite axial differential in indophenol blue reaction, except in the adhesive foot disc in which the reaction occurs a little more rapidly than in the remaining part of stalk.
3. In pieces with distal and proximal section made at the same time, the indophenol blue reaction occurs at the same time at both ends, progressing toward the middle, and the last part to show the reaction is the



middle of piece, i. e., the piece shows a typical bipolar gradient. In these pieces, a calyx invariably develops at both ends almost at the same rate and with the same scale of reorganization.

4. When the original calyx is present, the indophenol blue reaction in earlier stages of reconstitution appears quite uniformly over the whole length of the stalk, as in intact animals. And the presence of the original calyx also invariably prevents the development of a new calyx at the proximal cut end of the stalk.

5. In later stages of reconstitution with the original calyx present, somewhat more rapid reaction of indophenol blue appears in the extreme proximal stalk region. In the light of results with much later stages, it may be regarded as indicating an inhibited proximal gradient and as concerned with development of a basal disc.

6. When removal of the original calyx is delayed up to 72 hours after the time of proximal section, the unipolar frequency continuously increases from 0 to 85 per cent as indicated by indophenol blue reaction and from 0 to 95 per cent in reconstitution.

7. With delay of proximal section up to 72 hours after removal of the original calyx, the frequency of unipolarity increases from 0 to 95 per cent as indicated by indophenol blue reaction, and, from 0 to 100 per cent in reconstitution of calyx.

8. If distal and proximal sections are made at the same time, and a second distal section, removing the distal calyx primordium, is made with different periods of delay, the proximal calyx becomes increasingly dominant, and, as the result of control by this proximal dominance, development of a distal calyx and a distal gradient are inhibited. With 96 hour delay of the second distal section frequency of complete axial reversal increases to 80 per cent both in indophenol blue reaction and in reconstitution.

9. The region where a calyx will develop is usually distinctly visible about 5 hours after section as a region of most rapid coloration by indophenol blue with a gradient of decrement in coloration, which extends over a part or the whole length of the stalk piece. The gradient or gradients of indophenol blue reaction can be seen 3 or 4 days before the enlargement indicating calyx development is distinguishable.

10. The general parallelism in direction and frequency between polarities indicated by indophenol blue reaction and by reconstitution is so strikingly evident that the relation between the physiological gradient and the morphological polarity of reconstitution is beyond question.

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# STUDIES ON THE GROWTH HORMONES OF PLANTS

## III. THE OCCURRENCE OF GROWTH SUBSTANCE IN ISOLATED ROOTS GROWN UNDER STERILIZED CONDITIONS. (PRELIMINARY REPORT)

By

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Although it is an established fact that the growth substance is present in root tips, the question whether it is produced in them or not still remains unsettled. THIMANN (1934) supposed that the growth substance is not produced in the root tips but is merely transferred there. In opposition to THIMANN's conclusion, the writer (NAGAO 1936) has shown that the root tips themselves actually produce the growth substance. On this point, BOYSEN JENSEN (1936) and VAN RAALTE (1936) have come to the same conclusion as the writer.

On the other hand, FIEDLER (1936) has reported that, in the case of *Zea Mays* and of *Pisum sativum* the isolated root tips, cultivated in a suitable culture medium under sterilized conditions, continue to grow and are normally geotropic but that no growth substance is detectable in these roots. From the result of his experiments, he again dissented from the view that the growth substance is produced in the root tips, and supported THIMANN's conclusion.

In the present work, the writer has re-investigated the problem of the growth substance in isolated root tips cultivated under sterilized conditions.

### MATERIALS AND METHODS

*Helianthus annuus* was used as the material plant. The culture of the isolated root tips was made as follows:— After the pericarps had been removed, the seeds were soaked in tap water for 30–40 min., then sterilized in about 3%  $H_2O_2$  for 15–20 min. and washed in sterilized tap water. These seeds were germinated in test tubes on sterilized sawdust. When the roots were 2–5 cm in length, tips about 1 cm long were cut off, and introduced under sterilized conditions into test tubes containing a nutrient. A single root-tip was placed in each test tube.

The nutrient medium was prepared after WHITE (1934) and was the

same as that of FIEDLER (1936). It contained the following salts in 1000 ccm of distilled water:

Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	142 mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	74 mg
KNO <sub>3</sub> .....	81 mg
KCl .....	65 mg
KH <sub>2</sub> PO <sub>4</sub> .....	12 mg
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	3 mg

To this 20 g of dextrose and 10 g of agar were added. Extract of yeast, however, was not added.

The cultures were kept in a dark place, but were brought into the light at times to inspect the state of the growth. The temperature during the cultivation was 19°C–22.5°C in experiment 1, and 20.5°C–23.5°C in experiment 2.

The growth substance in the cultured root tips was extracted by BOYSEN JENSEN's dextrose-agar-method (1933), and its amount was determined by the modified *Avena* technique of WENT. [Cf. NAGAO, 1936. However, in the present experiments, the volume of the agar blocks was 2 cmm (2 × 1 × 1 mm) instead of 4 cmm, and the temperature, during the test of the growth substance, was 25°C instead of 23°C.] The extraction was made in the dark.

## RESULTS

On various days after the beginning of the culture, tips 1.5–2 mm long were cut off from the cultivated roots, and the growth substance in the tips was tested. The results are shown in Table 1.

TABLE 1.

	Duration of culture (days)	Average length of roots used (mm)	No. of root tips per agar block	Time on agar blocks (hrs.)	<i>Avena</i> curvature* (°)
Exp. 1	3	32	2	4	11.4 ± 1.3
	6	46	2	4	17.4 ± 1.8
Exp. 2	2	26	2	4	22.2 ± 1.0
	4	42	1	4	14.6 ± 1.0
	6	48	1	3	16.8 ± 1.0

\*Mean of 6 plants.

In the control agar blocks no growth substance was detectable.

In the case of *Helianthus annuus*, so far as the present experimental conditions were concerned, a considerable amount of growth substance was always found in the tips of the isolated roots during the 6 days of the culture. These results strongly suggest that the growth substance found in root tips is actually produced in the root tips themselves, and is not merely transferred from other parts of the plants.

The above facts do not agree with the results obtained by FIEDLER (1936) in the case of *Zea Mays* and of *Pisum sativum*. It is unsettled whether the disagreement is caused by the difference in materials or in method. Further experiments are now in progress.

The writer wishes to express his hearty thanks to Prof. Dr. Y. YAMAGUTI for his kind instruction in the course of this investigation.

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# EXPERIMENTAL STUDIES ON THE FUNCTION OF THE ANTERIOR HYPOPHYSIS

## I. INDUCED SEXUAL ACTIVITY IN THE FROGS

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(With two figures)

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Ovulation in adult amphibians has been experimentally induced by hypophyseal implants or by administration of extracts of the hypophysis in a number of species by various workers.

The present investigation was undertaken to determine the effect of daily implants of the anterior hypophysis and injection of the anterior hypophysis extracts in the Japanese frogs, *Rana japonica* GUENTHER and *Rana nigromaculata nigromaculata* HALLOWELL, and the results of the experiment are shown in this report.

The writer wishes to gratefully acknowledge indebtedness to Professor HATAI for helpful suggestions and criticism during the course of the work.

### MATERIAL AND METHOD

The adult frogs used in the experiment were obtained from the suburbs of Sendai. In this vicinity, *Rana japonica* G. lays eggs usually at the beginning of March, and *Rana nigromaculata* H. at the beginning of May.

In general frogs were kept for two or three days before use in an aquarium. The frogs were neither fed before nor during the experiment throughout.

In making the implantation the head of the donor was cut from the body with scissors, the brain exposed and the anterior hypophysis was removed from the floor of the cranium and inserted into the lateral lymph sac of the recipient.

The hypophysis-extract is prepared by grinding the anterior hypophysis with a glass rod in the bottom of a test-tube. One hypophysis is used in 1 cc. of 10% alcohol, and the extract was injected directly into the coelomic cavity of the frog, but in all of the following experiments the implantation rather than the injection method was used. After implanta-



tion and injection, the animals (5-7 pairs of frogs at a time) were placed in a wire-netting case, 45 cm. long, 25 cm. wide and 23 cm. deep, bottomed with iron plate through which a slow stream of water was run.

## RESULTS AND DISCUSSION

### 1) *Induced amplexus in the adult frogs.*

Male frogs, *Rana nigromaculata nigromaculata*, became activated following daily implants of the anterior hypophysis and injection of hypophysis-extracts for from 1 to 6 successive days, i.e. amplexus could be experimentally induced during a non-breeding season by the above-mentioned treatment. From this experimental work it has been found that the number of implants and dose of extracts necessary to bring about amplexus vary in individuals. The results are shown in tables 1-4.

TABLE 1.

*Induced amplexus in the frog, using the hypophysis  
from female frogs only.*

Experiment No.	Date, 1936	Water temp. °C	Number of hypophysis implanted	Results: Occurs hrs. after implantation
23	Oct. 16	17	2	Amplexus 1st day (3 hrs)
24	Oct. 16	17	2	Amplexus 1st day (3 hrs)
25	Oct. 18	15.5	2+2	Amplexus 3rd day
26	Oct. 19	16	2+2+2	Amplexus 4th day
27	Oct. 23	14	2	Amplexus 1st day (5 hrs)
28	Oct. 23	14	2	Amplexus 2nd day
29	Oct. 24	14	2+2	Amplexus 3rd day
30	Oct. 24	14	2+2	Amplexus 3rd day
31	Oct. 23	14	(Muscle)	Negative result
32	Oct. 23	14	(Muscle)	Negative result
33	Oct. 23	14	(Muscle)	Negative result

In tables 1-3, implantation method is used and materials are, both recipient and donor, *Rana nigromaculata nigromaculata*.

In this table and also in others, one or more hypophysis or 1 cc. of hypophysis-extract was administrated when amplexus did not occur 12 hours after treatment.

TABLE 2.

*Induced amplexus in the frog, using the hypophysis  
from male frogs only.*

Experiment No.	Date, 1936	Water temp. °C	Number of hypophysis implanted	Results
18	Oct. 14	17	2+2	Amplexus 3rd day
19	Oct. 15	17	2+2+2	Amplexus 4th day
20	Oct. 15	17	2+2+2	Amplexus 4th day
21	Oct. 16	17	2+2+2	Amplexus 5th day
22	Oct. 16	17	2+2+2	Negative result
34	Oct. 27	14	2	Amplexus 2nd day
38	Oct. 26	14	2+2+2 (testes)	Negative result
39	Oct. 26	14	2+2+2 (testes)	Negative result
40	Oct. 26	14	2+2+2 (testes)	Negative result
46	Nov. 5	12.5	2	Amplexus 2nd day
51	Nov. 14	10	3+2	Amplexus 3rd day
52	Nov. 16	10	3+2	Amplexus 5th day
62	Dec. 10	10.5	3+2	Amplexus 3rd day

TABLE 3.

*Induced amplexus in the frog, using the hypophysis  
from male and female frogs.*

Experiment No.	Date, 1936	Water temp. °C	Number of hypophysis implanted	Results
35	Oct. 30	14	8(2♂+2♂+2♂+2♀)	Amplexus 5th day
36	Oct. 31	14	8(2♂+2♂+2♂+2♀)	Amplexus 6th day
37	Nov. 3	14	12(2♂+2♂+2♂+2♀+2♀+2♀)	Amplexus 9th day
47	Nov. 6	11.5	4(2♂+1♂+1♀)	Amplexus 3rd day
48	Nov. 6	11.5	4(2♂+1♂+1♀)	Amplexus 3rd day
49	Nov. 7	12.5	6(2♂+1♂+1♀+2♀)	Amplexus 4th day

TABLE 4.

*Induced amplexus in the frog, using the hypophysis-extracts and testis-extracts.*

Experiment No.	Date, 1936	Water temp. °C	Number of injection required in cc.	Results
41	Nov. 8	12.5	1+1+1+1 (hypophysis)	Amplexus 5th day
42	Nov. 9	11	1+1+1+1+1 (hypophysis)	Amplexus 6th day
43	Nov. 4	13.5	1+1+1+1+1 (hypophysis)	Negative result
44	Nov. 4	13.5	1+1+1+1+1 (hypophysis)	Negative result
45	Nov. 4	13.5	1+1+1+1+1 (hypophysis)	Negative result
47	Nov. 8	12.5	2+2 (testes)	Amplexus 1st day
50	Nov. 8	12.5	2+2 (testes)	Amplexus 1st day
53	Nov. 20	10	1+1+1 (testes)	Amplexus 7th day
54	Nov. 20	10	1+1+1 (testes)	Negative result
55	Nov. 20	10	1+1+1 (testes)	Negative result
56	Nov. 20	10	1+1+1 (testes)	Negative result
57	Nov. 20	10	1+1+1 (testes)	Negative result

In this table, injection method is used and materials are, both recipient and donor, *Rana nigromaculata nigromaculata*.

### 2) Induced ovulation, oviposition and amplexus in the adult frogs.

In December, *Rana japonica* gave a positive response to the treatment by spawning several dozen eggs fertilized and by ovulating many eggs in uteri (Fig. 1). And also, treated female frogs ovulated in uteri and laid eggs without amplexus (Fig. 2).

At the middle of February, in *Rana nigromaculata nigromaculata* ovulation and oviposition could be induced, and also ovulation and oviposition are successfully attempted when the females are alone sexually stimulated. The results are shown in table 5.

### 3) Artificial fertilization.

After the implantation and injection the female frogs (*R. japonica* and *R. nigromaculata*) are kept at room temperature and after 2 or 3 days when the eggs were ovulated in uteri, they are stripped directly into a dish containing a thin layer of sperm suspension at room temperature and then fertilization occurs easily. Thirty minutes after insemination the dish may be filled with tap-water.

The sperm suspension is prepared by macerating two testes of untreated frogs (*R. japonica* and *R. nigromaculata*) in 10 cc. of tap-water and is allowed to stand for at least ten minutes before using.

The fertilized eggs hatched out after six days at 16-17°C (water-temperature) and developed to normal tadpoles.

The present experiment was done during the period extending from September 1936 to February 1937 and in all (ten) cases, amplexus was unsuccessfully attempted during September to early October. Ovulation and oviposition in *Rana japonica* were successful for the first time in December and in *Rana nigromaculata nigromaculata* in February, namely in both species of *Rana* ovulation and oviposition occurred about three months earlier than that of normal in the breeding season.

From the above-mentioned, it is suggested that even by hypophyseal implants and by injection of hypophysis-extracts amplexus, ovulation and oviposition can not be induced so far as the frog's gonads are not matured to a certain degree.



Fig. 1. Experimentally ovulated (left) and controlled (right) frogs (*Rana japonica* G.). Natural size. n, normal ovary; o, ovary (almost empty); u, uterus filled with eggs.

The period of amplexus in the experiment was from one to three weeks and does not depend upon the dose of hypophysis.

During the experiment four (male) frogs gave a negative response to the hypophysis treatments. These testes were extremely small compared with those of others.

The fact that amplexus could be induced by injection of testes-extracts of the frogs suggests an interesting question of hypophyseal-gonadal interrelation.

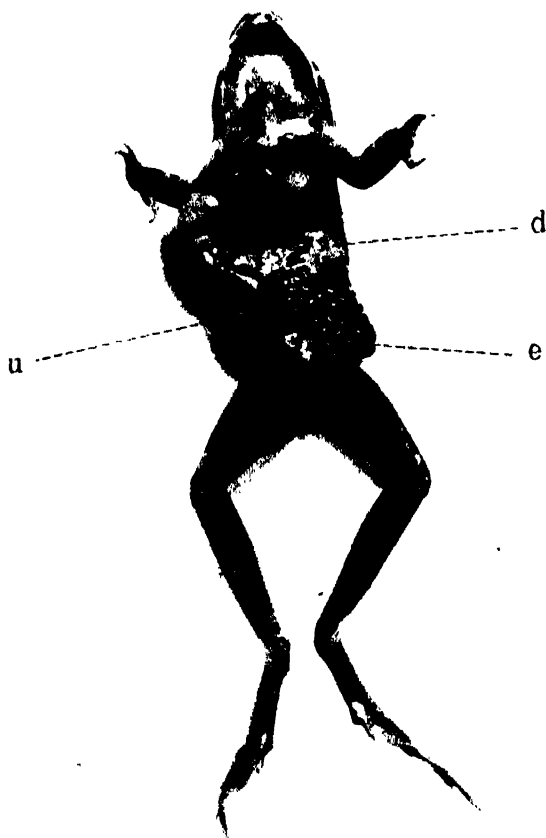


Fig. 2. Experimentally ovulated frog (*Rana japonica* G.). Natural size. d. an egg in the oviduct; e. eggs taken from the uterus, showing deposition of jelly; u. uterus filled with eggs.

TABLE 5.

*Induced amplexus, ovulation and oviposition in the frog, using the hypophysis from male and female frogs.*

Experiment No.	Date '86-'87	Water temp. °C	Recipient	Donor	Number of hypophysis implanted	Results
63	Dec. 13	9.5	R. jap.	R. nig.	2♀+3♀	Amplexus 3rd day, 20 eggs laid (unfert.) 3rd day
64	Dec. 13	9.5	R. jap.	R. nig.	2♀+2♀	Amplexus 3rd day & ovulation 3rd day
65	Dec. 19	10	R. jap.	R. nig.	2♀+(1♂+1♀)	Amplexus 4th day & ovulation
66	Dec. 20	12	R. jap.	R. nig.	2♀+(1♂+1♀)	Amplexus 5th day & many eggs in uteri
67	Dec. 22	10	R. jap.	R. nig.	2♀+(1♂+1♀)	Amplexus 7th day & 805 eggs in uteri
68	Dec. 17	12	R. jap.	R. jap.	2♀	Amplexus 2nd day & 796 eggs laid (fert.) 10th day
69	Dec. 19	10	R. jap.	R. jap.	2♀+(1♂+1♀)	Amplexus 4th day & 1180 eggs laid (fert.) 10th day
70	Jan. 5		R. jap.	R. jap.	(2♂+1♀)+(1♂+1♀)	Amplexus 9th day & ovulation
71	Jan. 6		R. jap.	R. jap.	(2♂+1♀)+(1♂+1♀)	Amplexus 10th day & ovulation
72	Jan. 9		R. jap.	R. jap.	(2♂+1♀)+(1♂+1♀)	Amplexus 13th day & ovulation
76	Jan. 11		R. jap.	R. nig.	1♂+1♂+1♀	ca. 50 eggs laid without amplexus
77	Jan. 11		R. jap.	R. nig.	1♂+1♂+1♀	ca. 50 eggs laid without amplexus
78	Feb. 13	6	R. nig.	R. nig.	2♂+2♂	50 eggs ovulated without amplexus
79	Feb. 13	6	R. nig.	R. nig.	2♂+(1♂+1♀)	3 eggs in oviduct & 50 eggs in uteri (10th day)
80	Feb. 13	6	R. nig.	R. nig.	2♂+(1♂+1♀)	ca. 50 eggs in uteri & oviposition (10th day)
81	Feb. 13	6	R. nig.	R. nig.	2♂+(1♂+1♀)	Ovulation & oviposition (10th day)
82	Feb. 13	6	R. nig.	R. nig.	2♂+(1♂+1♀)	Laid few eggs without amplexus
83	Feb. 13	6	R. nig.	R. nig.	2♂+(1♂+1♀)	Laid few eggs without amplexus
84	Feb. 13	6	R. nig.	R. nig.	2♂+(1♂+1♀)	Laid few eggs without amplexus
85	Feb. 13	13	R. nig.	R. nig.	1♀+1♂	Amplexus & 15 eggs laid, ca. 700 eggs in uteri
86	Feb. 21	11.5	R. nig.	R. nig.	(1♂+1♀)+(1♂+1♀)	Amplexus & ca. 500 eggs in uteri, ovaries almost empty

Experiment No.	Date '36-'37	Water temp. °C	Recipient	Donor	Number of hypophysis implanted	Results
73	Jan. 10		R. jap.	R. nig.	1+1+1 cc. (hypophysis)	Amplexus 13th day & ovulation
74	Jan. 11		R. jap.	R. nig.	1+1+1 cc. (..)	Amplexus 14th day & ovulation
75	Jan. 11		R. jap.	R. nig.	1+1+1 cc. (..)	Ovulation without amplexus

In this table, implantation method was used except Nos. 73-75.

### CONCLUSIONS

1) Amplexus, ovulation and oviposition were possible to be induced during a non-breeding season by implantation of the anterior hypophysis and by injection of the anterior-hypophysis-extracts.

2) Amplexus is successfully attempted when the male is alone sexually stimulated.

3) Ovulation and oviposition are successfully attempted when the female is alone sexually stimulated.

4) Amplexus were possible to be also induced by injection of the testes-extracts of the frogs.

5) Artificial fertilization in the frogs treated with the anterior hypophysis takes place easily to develop to a normal tadpole.

6) There is no sex-specificity in the function of the anterior hypophysis.

7) Period of amplexus is from one to three weeks.

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# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## VII. ON THE FORMED ELEMENTS IN THE BODY FLUID OF SOME MARINE INVERTEBRATES WHICH POSSESS THE RED BLOOD CORPUSCLES<sup>1)</sup>

By

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(With twenty-four figures)

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In the present paper, observations of the formed elements in the blood and perivisceral fluid of

1. three gephyrean worms;  
*Urechis unicinctus* (VON DRASCHE),  
*Thalassema gogoshimense* IKEDA,  
*Physcosoma scolops* (SELENKA et DE MAN),
2. a polychate worm;  
*Terebella* sp. (*Terebella debilis*?),
3. two mussels;  
*Arca inflata* (REEVE)  
*Glycimeris vestitus* DUNKER,
4. two holothurians;  
*Caudina chilensis* (J. MÜLLER),  
*Molpadia rotetzii* V. MARENZELLER,

are reported together as a matter of convenience. For all of these animals possess the red colored corpuscles in their body fluid, and to compare the structures of the red cells of these different animals seemed to be of interest. Of the specimens here used *Thalassema* and *Glycimeris* were collected from the Gogoshima Island, Ehime Prefecture; most of *Urechis* were gathered from Hiroshima bay, Hiroshima Prefecture. A part of *Urechis* and all the other animals were obtained from the vicinity of the Asamushi Marine Biological Station.



## OBSERVATIONS

## Part I. Red Coloured Corpuscles.

*Urechis uncinatus*.

The body fluid is readily drawn off from the perivisceral cavity by means of a small hypodermic needle. The fluid is usually scarlet in colour due to the presence of numerous red blood corpuscles which contain haemoglobin. Under microscopic examination of the body fluid the cytoplasm of red corpuscles appears pale yellow with haemoglobin, and numerous, colorless and highly refractory granules which are uniform in size (about  $1\mu$  in diameter) and spherical in shape are scattered uniformly or

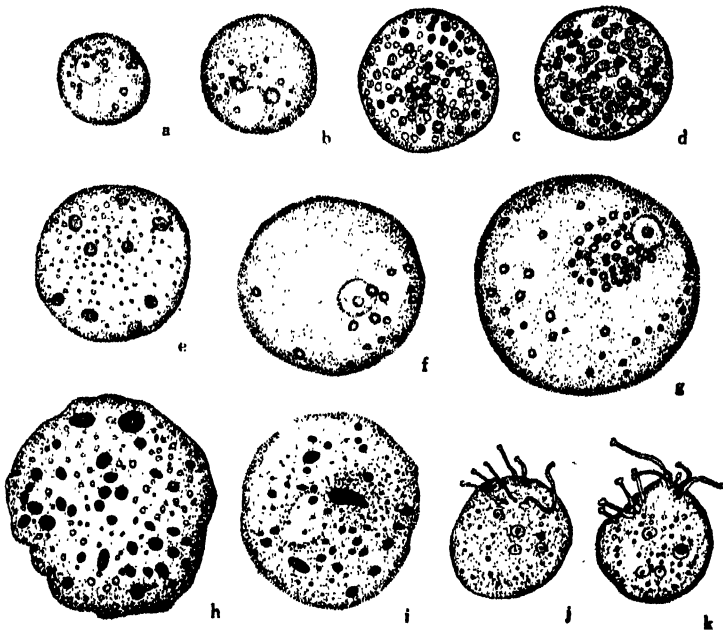


Fig. 1. The red blood cells of *Urechis uncinatus*. ca. 1,000 $\times$ .

a-g. Fresh and unstained corpuscles.

h, i. Corpuscles impregnated with silver.

j, k. Corpuscles with pseudopodia-like protuberances.

conglomerated tightly in the perinuclear region (Fig. 1). The red corpuscles are usually spherical and slightly biconvex disks with smooth contour and round edges. Cells measured in the state of suspension show a good deal of variation in size such as 10–36  $\mu$  in diameter and 3–6  $\mu$  in thick-

ness. Owing to the density of haemoglobin which is distributed throughout the cell, the nucleus is, at first, usually invisible or can hardly be distinguished as a small, clear and circular area in fresh and unstained preparations, but it becomes clearer if one keeps the cells under observation for a while. The nucleus is small and round, and located eccentrically. The nuclear contents are not discernible in unstained preparations except the constant presence of a single or double nucleoli. The bi- or polynucleism of red cells which is occasionally seen in the blood of *Sipunculoidea* was not found.

The red cells appear to be surrounded by a remarkably strong cell membrane, which does not burst in a hypotonic (0.7% NaCl) or a hypertonic (8% NaCl) solution. In the swollen corpuscles, the granules exhibit active Brownian movement, suggesting the fluid state of the cell interior. In the slightly hypertonic sea water, several processes which are similar to the tube feet of echinoderms are seen on the surface of cell (Fig. 1, j, k). With an increase of concentration of sea water, these processes become numerous, and cells grow to so-called thorn-apple shape, the same as the red blood corpuscles of mammals.

The resistance of cell membrane to saponin, however, is rather weak. Saponin solution for the test is prepared with normal sea water. When immersed in a 0.003% solution of saponin, the cells swell gradually, and their contour becomes erosive. Within a few hours the membrane ruptures spontaneously, and the granules which were confined in the cell and remained with no remarkable change or growing into a few large granules by fusing with each other, flow out through the localized opening.

By the repeated intraperitoneal injections of neutral red, usually a single, occasionally two or three granules which recall the segregation apparatus of the vertebrate-erythrocytes appear in the perinuclear region. These granules appear also by the supravital application of neutral red, brilliant cresyl blue, Nile-blue sulphate, methylen blue, etc.

In a preparation, for example, the number of cells containing a single neutral red body was 104, that of two was 33, and that of three 12. The appearance of secondary induced granules needs long exposure to dye, and there is found no induction granules within three hours exposure.

When the red cells are exposed supravitaly to Janus green B, numerous granular or fibrillar mitochondria which are distributed irregularly throughout the cell appear within one or two hours. Granular substance which fills up the cell is also demonstrable by using vital dyes. SABIN's method was followed in the supravital application of various dyes, and

dilution of dyes was always 1:1,000.

The refractive granules in the cytoplasm can also be stained supravitaly with various dyes. The granules begin to be stained with Nile-blue sulphate within 30 minute-exposure. In the case of staining with brilliant cresyl blue, Janus green B and methylen blue, it needs about one hour. To stain the granules with neutral red, two hours or more exposure is necessary. The granules are stained deep red with neutral red, greenish blue or bluish purple with Nile-blue sulphate, Janus green B and methylen blue. The most striking staining patterns are produced with brilliant cresyl blue applied in concentrations high enough to stain the nucleus (Fig. 2). The granules appear light blue, purple or pink; newly induced granules, rods or filaments are deep blue or purple; the vacuoles which also newly appeared in the course of supravital staining are pink; and

the nucleus is light blue. In the center of vacuoles a deep blue particle which shows active Brownian movement is usually visible. With a close examination, it is clear that the granules are stained heterogeneously; the surface layer of granules is blue and the rest pink. The different depths of microscopic focusing may be the cause, therefore, of various shades of

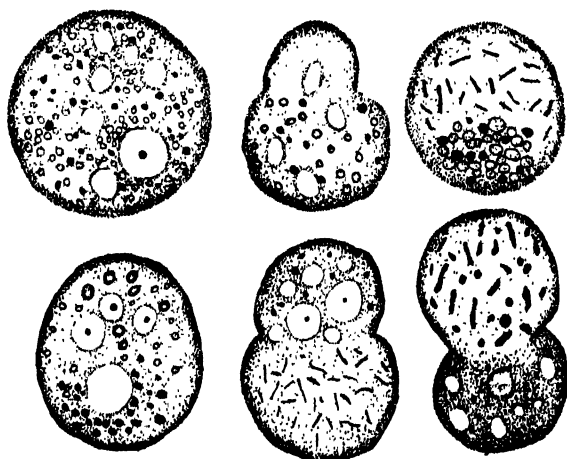


Fig. 2. Fresh corpuscles of *Urechis* stained supravitaly with brilliant cresyl blue, showing the basophilic bodies (black) stained deep blue, the clear globules stained pink, and the rods or filaments stained violet. ca. 1,000 $\times$ .

granules. The newly formed vacuoles owing to the action of brilliant cresyl blue are, I think, the granules which fuse with each other and grew into larger ones. The pink shade of vacuoles probably comes from that of central part of granules, and blue particle in the middle of vacuoles originates from the surface layer of granules.

On the nature of these refractive granules, the haematologists have paid but little attention till the present time. In their extended study

of haemoglobin of *Urechis caupo*, REDFIELD and FLORKIN (1931) did not refer to the property of the granules. BAUMBERGER and MICHAELIS (1931) described that these granules have "a rather high refractory index, though not so high as that of fat drops". In the present specimens that the granules are composed essentially of a fatty substance is evident from the following facts. The granules are soluble in alcohol, ether, chloroform, benzol, acetone, various alkali and so forth immediately. They resist relatively to acids and are not soluble in cold distilled water. They are stained intensely in the characteristic shades of fats with Nile-blue sulphate, brilliant cresyl blue, and especially Sudan III. The staining with the latter was made after the fixation of cells with formol. Osmic acid causes the blackening of the granules.

Besides the fatty substance the granules contain some other components. The granules are positive to the indophenol blue synthesis. Using SATO's method, peroxidase is also detected in the granules, but this reaction is less intensive than that of oxydase. By treating with antimony trichloride they assume a shade of indigo blue occasionally, showing the presence of vitamin A. The granules are also positive to silver impregnation (Fig. 1, h, i). Iron detection of granules was negative.

In addition to these granules, there are many of a brown pigment in the red corpuscles of some specimens (Fig. 1, c-e). The occurrence of pigmented granules is more abundant in the animals whose blood appears from brownish red to dark brown rather than scarlet. It was made clear by BAUMBERGER and MICHAELIS in the study of the blood pigments of *Urechis caupo* that this pigment is the haematin, and the most dark-coloured blood is met with in large, sex-matured animals.

The fine cellular structures which are positive to silver impregnation is demonstrated in the erythrocytes of many vertebrates. The present author applied FUJITA's method or its modification (TOMITA and others, 1934) to the red corpuscles of *Urechis*, and found that many fine or coarse, impregnated granules appear in the cytoplasm. Most of these granules, however, were those mentioned above, and cytoplasmic inclusion similar to vertebrate-erythrocytes was not found at all.

In dry-fixed smears stained by GIEMSA's method, the granules are basophilic, and appear as reddish purple bodies. The large vacuoles, when they are present, remain clear and uncoloured. The nucleus is small, spherical and chromatic, and usually eccentrically located. The nucleoli are stained pink in colour by KUNI's method.

It has long been recognized that haemoglobin rarely crystallizes within

the red blood corpuscles of vertebrates. DAWSON observed it in the erythrocytes of an amphibian, *Necturus* (1930), and a fish, *Syngnathus fuscus* (1932). He reviewed this subject in his paper (DAWSON, 1932) and pointed out the deficiency of references. In the present investigation

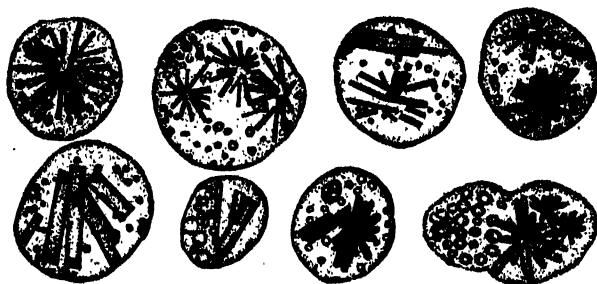


Fig. 3. Haemoglobin crystals within the red cells of *Urechis*. ca. 900 $\times$ .

this phenomenon was accidentally observed. For the purpose of supravital staining with neutral red, Janus green B, etc., I made preparations of red cells after the method of SABIN. They were allowed to stand overnight. On the next morning I found that the cells contain numerous, small or large columnar crystals, aggregating in the shape of rosette. Occasional cells contain three or more of such rosettes, and the outline of cells, accordingly, becomes irregular. The shade of supravitally applied dyes is usually faded as a result of the intracellular reduction. In the fluid immersing the red cells there are found also tetragonal bipyramid crystals of haemoglobin. DAWSON (1932) stated that little is known of the factors involved in maintaining the haemoglobin within the red blood cell in solution, and that the only cause of the intracellular crystallization of haemoglobin in the erythrocyte is the slow withdrawal of water. In the present instance there is no additional fact to his description.

#### *Thalassema gogoshimense.*

There is no fundamental difference of the red blood corpuscles between *Urechis* and *Thalassema*. The red blood corpuscles of *Thalassema* are sensitive to the change of environmental factors. They assume always irregular shapes with protruding lobular pseudopodia-like protuberances from the cell surface, though the microscopic examination was made immediately after a withdrawal of body fluid (Fig. 4). When one used, however, a very rapid fixative such as I-Ik, BOUIN's solution, dilute osmic

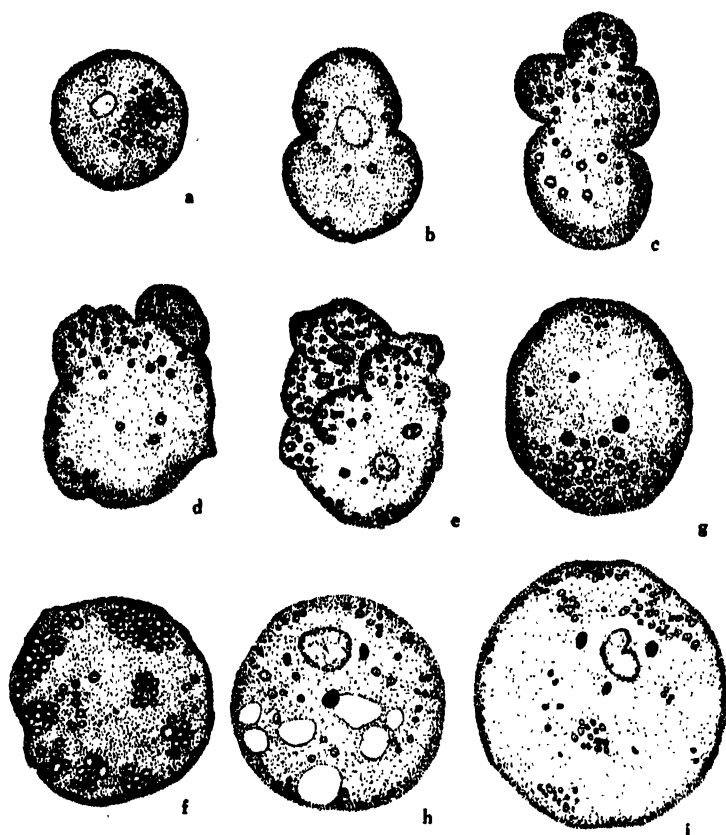


Fig. 4. The red cells of *Thalassema*. ca. 1,000 $\times$ . Fresh corpuscles stained with neutral red. Black bodies show the segregation apparatus.

acid, etc., they remain constantly spherical disks with smooth, circular outline. The size and shade of red blood cells are similar to those of *Urechis*. The small, round oval or kidney-shaped nucleus is located eccentrically and is usually obscured with diffusely scattered haemoglobin, in fresh and unstained preparations. I failed to detect the anucleated erythrocytes (erythroplastids) which were found by ROMIEU (1923) in a worm, *Magelona papillicornis*. The nucleolus is rather infrequently found in this specimen while it was a constant element of nucleus in the red blood cells of *Urechis*. The neutral red bodies are three or four in number and located in the perinuclear region as a single or two clusters (Fig. 4, g-i).

Preformed, colourless and high refractory granules aggregated into

several clusters, and properties of these granules to various dyes and reagents are similar to those of *Urechis*. Brown pigment is also found in the present specimens.

*Physcosoma scolops.*

Small as this worm is, it has relatively voluminous body cavity and contains a large amount of body fluid in it. The fluid is a pale rose in colour, due to the presence of numerous erythrocytes which contain haemerythrin and suspended in the colourless fluid. The corpuscles are pale yellow when viewed separately, and are spherical or oval disk, more or less biconvex (Fig. 5, h, i). They assume, however, shapes of spindle, cap, trigon, tetragon and so forth in vitro, owing to the change of external conditions (Fig. 5, a-g). Size of red blood cells varies considerably even in the same individual; namely  $8-20\mu$  in diameter and  $2-4\mu$  in thickness. The nucleus, usually obscured in fresh, unstained preparations due to the density of the haemerythrin, becomes visible when one keeps the corpuscles under the microscopic examination for a while. The nucleus is usually eccentrically located, and is round or oval in shape, but kidney- or cocoon-shaped ones are encountered occasionally. The size of nucleus

exhibits also a good deal of variation; the smaller type is about  $2-4\mu$  in diameter and contains relatively small amount of chromatin while the larger type is  $6-8\mu$  in diameter, and a large amount of chromatin is found in it (Fig. 6, a-i). The nucleolus is not obvious in either type of nuclei. The nucleus possesses an affinity to the neutral violet, and a considerable amount of chromatin granules appeared in it when this dye was applied supravitaly. The binucleated red cells are

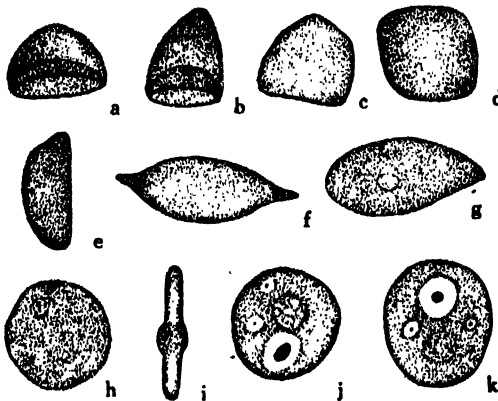


Fig. 5. The red cells of *Physcosoma*. ca.  $1,000\times$ .

a-h. Fresh and unstained cells.

i. The profile of a fresh cell.

j, k. Fresh cells stained supravitaly with brilliant cresyl blue showing the clear globules stained pink, the granules (black) in the globules stained deep blue.

found occasionally (Fig. 6, j-m), but tri- or polynucleated cells, and anucleated ones as well, were not encountered with.

Numerous fine granules, either colourless or light yellow, are seen frequently in the cells. They are active in Brownian movement. Large, colourless and more refractive globules are also recognized. Occasional cells contain colourless rhomboid crystals in cytoplasm (Fig. 7, a-c), and they exhibit also an active Brownian movement. The number of crystals is usually from one to several, but it reaches twenty or more upon rare occasions. The presence of such crystals in the red blood cells of *Sipunculus nudus* was described by METALNIKOFF (1899 and 1900) and KOLLMANN (1907), but there is no instance to be comparable with the present specimen in the abundance of crystals, so far as I am aware. These crystals remain irregular shaped, clear, or as slightly eosinophilic vacuoles in the dry-fixed smears stained by WRIGHT's or GIEMSA's

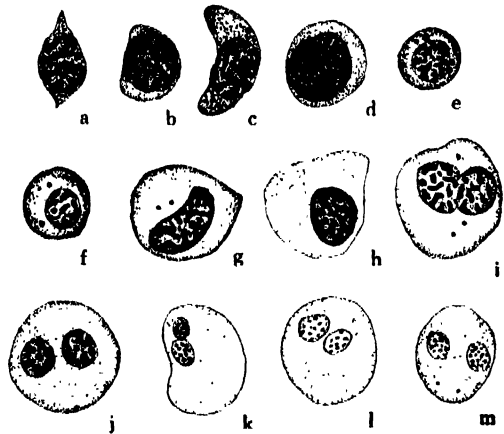


Fig. 6. The blood cells of *Physcosoma* stained by GIEMSA's method. ca. 1,000 $\times$ .

- a-e. Haemocytoblasts. The cytoplasm stains basophilically.
- f-i. Erythroblasts or young erythrocytes. The cytoplasm shows more or less polychromatophilism.
- k-m. Matured and binucleated erythrocytes.

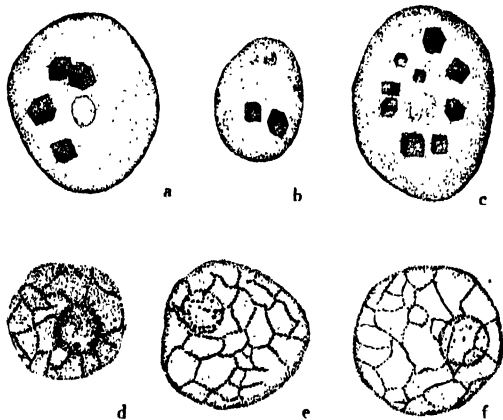


Fig. 7. The red cells of *Physcosoma*. ca. 1,100 $\times$ .

- a-c. The red cells with crystals. Fresh and unstained.
- d-f. The red cells impregnated with silver.



method. On the nature and function of the crystals nothing definite is known.

By the supravital staining with neutral red, methylen blue, Nile-blue sulphate, brilliant cresyl blue and so forth, from one to several stained granules appear immediately in the perinuclear region (Fig. 8, a-d). These are, perhaps, the refractory coarse granules mentioned just above, for there remains no large granule which is highly refractive and unstained. The fine granules begin also to be stained with these dyes soon later

(Fig. 8, e, f), and the large granules disappear gradually as the result of the dispersion into cytoplasm. When the nuclear staining is begun, the cytoplasm is filled loosely with fine and stained granules which are arranged in numerous and short rods (Fig. 8, g, h), and it is not possible to distinguish the difference between the primary and secondary granules, either on the basis of size, intensity of staining or

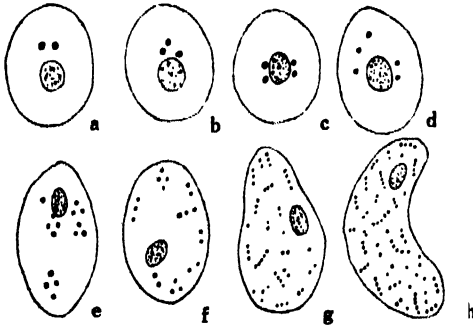


Fig. 8. Fresh red corpuscles of *Physcosoma* stained supravitally with neutral red. ca. 1,000 $\times$ .

The black points show the neutral red bodies (a-d) or the induced granules (e-h).

position within the cell. With Janus green B, the mitochondria which are of rod-shape and scattered throughout the cytoplasm or clustered in the perinuclear zone are demonstrated. Using brilliant cresyl blue the striking staining patterns which are seen in the red blood cells of *Urechis* and *Thalassema* are also recognized in the present specimen.

The coarse granules stainable with neutral red are blackened on prolonged exposure to osmic acid. They are basophilic, and show the positive reaction to silver impregnation and indophenol blue synthesis. Except the last one, these reactions are seen also in the segregation apparatus of vertebrate erythrocytes. It seems possible, therefore, to suppose that there is a close relation between these coarse granules and the segregation apparatus. There are occasional cells in which preexisting granules are not seen. In these cells the neutral red bodies appear after an adequate treatment. Accordingly, it is also supposed that these bodies exist in the red cells of *Physcosoma*, regardless of the visibility in fresh and unstained preparations. DAWSON (1932b), working with *Phascolosoma*

*gouldi*, stated that no induction or neoformation of bodies stainable with neutral red was obtained even after a long exposure to the dye. In the present case, however, the result is the reverse, and numerous, fine and stained granules, which far exceeded in number the preexisting fine granules, are seen in the cells under prolonged exposure. It was not possible to recognize the difference between preexisting and induced granules.

The silver impregnation was made after the method of TOMITA and others. The result was very satisfactory as is seen in Fig. 7, d-f. The cell body is stained brownish yellow, and the nucleus is stained also in the same tone, but more or less deeper than the cytoplasm. The nuclear membrane is distinctly discernible while the nuclear contents are almost homogeneous and any definite structure is not visible. In about one third of red cells conspicuous patterns are encountered. These patterns are a coarse-meshed network whose branches tend to radiate from the nucleus to the periphery of cells. With a close microscopic examination it is evident that the network is composed of numerous, fine argentophilic granules. TOMITA and his co-workers (1934) made systematic studies on the minute structures of erythrocytes of different classes of *Vertebrata* with their silver impregnation method. These studies have led to the conclusion that there is a structure characteristic of every species, and that the lower the class to which an animal belongs the more complex is the silver impregnated structure of the red cell and vice versa. That the impregnation patterns of present case were very much similar to those of fishes and amphibia is rather a natural consequence. The immersion of red cells in a hypotonic salt solution (0.6% NaCl, for example) or a weak solution of saponin (0.001%) for an hour or two causes the disintegration of the reticular structure.

In the dry-fixed smears stained with GIEMSA's solution the preexisting granules are stained basophilically. The cytoplasm of matured red blood cells is, naturally, eosinophilic, but that of younger ones shows more or less polychromatophilism, and there is seen every gradation of cytoplasmic tint between eosinophilia and basophilia.

The nucleus is stained reddish purple in colour. Polychromatophilic red corpuscles possess usually a large nucleus, and the chromatin takes an arrangement of cart-wheel shape similar to that of vertebrate-erythroblasts (Fig. 6, a-j). The nucleus of matured red corpuscles is small in size, round, oval or kidney-like in shape, and chromatin granules are scattered irregularly throughout the nucleus (Fig. 6, k-m). By the KUNII's

method the nucleolus is occasionally detectable.

*Terebella* sp. (*T. debilis*?)

The body cavity of this worm contains haemolymph with numerous suspending corpuscles. Through the thin wall of gill-filaments the formed elements composed essentially of the red cells and floating reversibly to

and from are clearly visible. The fluid obtained by aspiration directly from the body cavity is scarlet in colour and contains occasionally a large amount of genital products. The corpuscles are pale rose in colour when they are examined one by one, and are usually oval, occasionally circular in outline (Fig. 9). When viewed on edge they are seen to be relatively thick, slightly bi-convex discs. The size of corpuscles shows a good deal of variation, measuring 8–25  $\mu$  in larger-, 6–14  $\mu$  in shorter diameter, and 2–5  $\mu$  in thickness. The haemo-

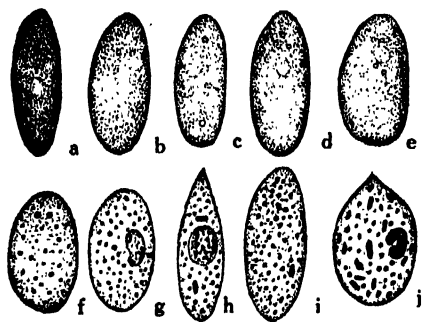


Fig. 9. The red cells of *Terebella*. ca. 1,000 $\times$ .  
a-e. Fresh and unstained corpuscles.  
f i. Fresh corpuscles stained with neutral red showing the induced granules.  
j. Fresh corpuscle stained with Janus green B showing the mitochondria.

globin is uniformly distributed throughout the cell in high concentrations sufficient to mask the nucleus completely. In the cytoplasm usually two to four, relatively large and high refractive globules, which are colourless or slightly yellowish, are clustered in the perinuclear zone (Fig. 9, a-e). These globules are readily and intensely stainable supravivally with neutral red, brilliant cresyl blue, Nile-blue sulphate etc. They are also positive to oxydase reaction and silver impregnation. Osmic acid blackens them slightly, but they are unstained with Sudan III and Scharlach R. Iron reaction is also negative. Besides these preexisting globules so called segregation apparatus is usually invisible. A long exposure of cells to the dye causes the appearance of secondary, perhaps, induced granules which fill up the cell body (Fig. 9, f-i). By the supravital staining with Janus green B the mitochondria are detected (Fig. 9, j). Silver impregnation was made, using the method of TOMITA and others. In most of the cells the globules mentioned just above were the only structures which

showed the positive reaction to the impregnation. But the cells filled up with numerous and fine argentophilic granules were encountered occasionally. The reticular structure such as seen in *Physcosoma* was not found in any specimen. The nucleus is relatively large, measuring  $4-6\mu$  in diameter and situated eccentrically. In the dry-fixed smears stained by GIEMSA's method the globules show the basophilic reaction. The chromatin granules are rather coarse and are arranged more densely in the marginal portion of nucleus than in the center. The nucleolus was not discernible. Occasional cells are binucleated, but the erythroplastid was not encountered absolutely.

### *Arca inflata.*

The blood of *Arca inflata* was the subject of SATO's elaborate investigations (1931). The present study was undertaken in desire to make some supplementary observations on the morphology of red blood corpuscles with special reference to the nature of brown granules which are found abundantly in the corpuscles.

Recently DAWSON (1932b) reported on the supravital staining of the coloured corpuscles of *Arca transversa*. As was described by him, Janus green B brings out also a variable number of small, perinuclear bodies which appear either as granules or short rods, in the present specimens. With brilliant cresyl blue the new appearance of numerous, fine granules was also observed. After a long exposure (10 hours or more) to dyes, from one to three granules which are remarkably large, regularly round, deep red in colour, and active in a quivering movement appeared with some frequency. Similar granules to these were seen also in a few cases of staining with Janus green B. Further investigation is necessary to determine whether these are so-called segregation apparatus or accidental products, for the appearance of the granules needs so long an exposure to dye, and the actual instances are not sufficient.

The preexisting brown granules are also stainable supravitally with brilliant cresyl blue, Nile-blue sulphate, Janus green B, methylen blue, neutral red, methyl green, etc. Most quick and intensive staining is obtained with the first two dyes. SATO reported the negative result of staining with Janus green. In the present case, however, Janus green B was one of appropriate dyes, except the fact that this dye is readily reduced in the anaerobic conditions, especially when it was applied as a very weak solution. The brown granules are irregular in shape in fresh

and unstained preparations. They become, however, circular in outline after long exposure to Nile-blue sulphate and others, as it was observed already by DAWSON. The granules in this condition tend to agglutinate into a cluster in the perinuclear region. As SATO described, application of osmic acid causes a blackening of the surface of granules, but Sudan III or Scharlach R. does not stain there. By the microscopic examination it is evident that the surface zone of granules is stained with vital dyes more deeply than the center. And it seems to me that the rounding up of granules may be due to the increase of this chromatophilic area.

With SATO and DAWSON the chemical nature of granules passed unnoticed. The granules are insoluble in alcohol, ether, chloroform or distilled water, but soluble in acidulated alcohol, acetic acid, mineral acids and various solutions of alkali. The presence of iron in these granules is detected also by Berlin blue reaction. From these reactions it is supposed that these granules contain haematin or a pigment closely related to it. The presence of free haematin in the red corpuscles is reported by BAUMBERGER and MICHAELIS in the blood of *Urechis caupo*, as it was mentioned above.

The brown granules exhibit a positive reaction of indophenol blue synthesis very intensely. It is evident, therefore, that these granules are a

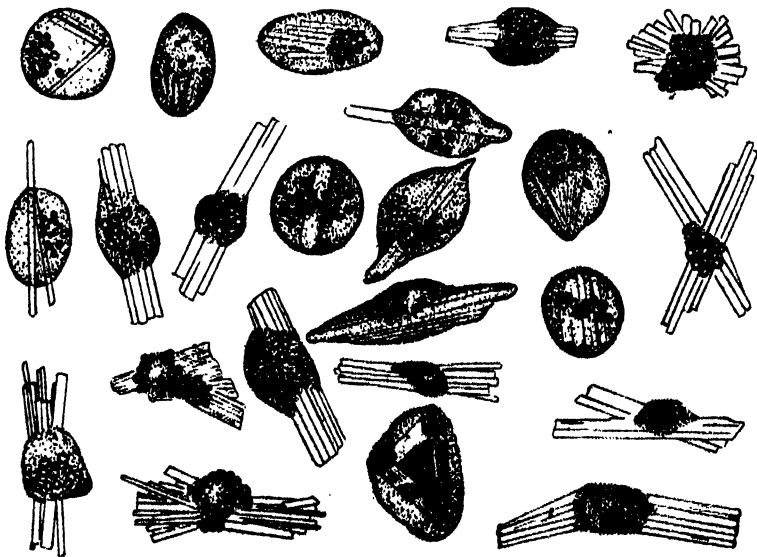


Fig. 10. Haemoglobin crystals within the red cells of *Arca*. ca. 900 $\times$ .

complex of various chemical components, and are not merely accumulated waste products.

As it was seen in the case of *Urechis*, the intracellular crystallization occurred also in the red cells of *Arca* by a chance of supravital staining with neutral red (Fig. 10). The crystals of oxyhaemoglobin of *Arca* are pale rose in colour, rhombic prisms in shape, as was described and photographed by SATO. Crystallization begins in the center of red cell as a short rod. The elongation of crystal progresses to the direction of long axis, and the outline of cell, accordingly, turns to a spindle shape. At last the crystal penetrates the cell membrane and both ends of it come to the outside of the cell. It occurs also frequently that many crystals are formed simultaneously in a cell. These crystals aggregate usually in parallel or radial orientation. Spindle, tri- or tetragonal arrangements of crystals are seen also frequently. The deformation of cellular outline is influenced by the form of the crystal-aggregation. In any case the cell membrane becomes invisible with the advance of growth of crystals, while the main parts of cells and preexistent granules stained in deep red with neutral red remain undissolved and adhering nearly at the middle portion of crystal-bundles.

In the dry-fixed smears stained by GIEMSA's method, a small spherical nucleus, generally eccentric, is visible. A large nucleus is encountered in those cells which have

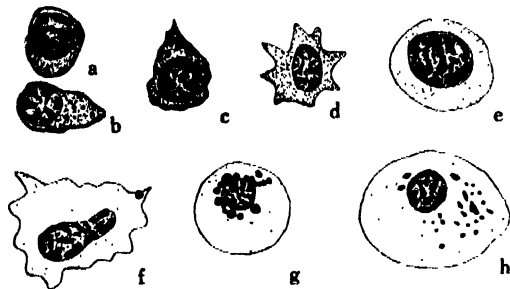


Fig. 11. The red cells of *Arca* showing various stages of development. (Stained by GIEMSA's method) ca. 1,100 $\times$ .

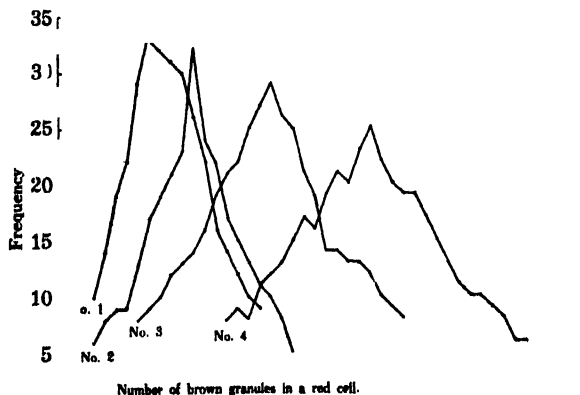


Fig. 12. Variation curves of the number of brown granules in a given red cell which were obtained from four individuals.

polychromatophilic cytoplasm and no or a few granules (Fig. 11, a-e). Chromatin of such nucleus assumes the arrangement of cart-wheel, though it may not be so typical. It is supposed that this type of cell may be young erythrocyte.

As SATO stated already, the number of brown irregular granules of the red corpuscles shows a remarkable fluctuation — from three to several dozens or more. In the red corpuscles of a given individual, however, the fluctuation exhibits more or less a smaller deviation. Table 1 and Fig. 12 show some results of the biometry of the number of granules in a given cell which were obtained from four individuals respectively.

TABLE 1.

*Biometric data of fluctuation of the brown granules in a red blood cell.*

Animals	Numbers of red cells	Maximum & minimum	Mean	Standard deviation	Coefficient of variability (%)
No. 1	357	3-19	$10.4 \pm 0.22$	4.1	39.4
No. 2	287	3-21	$12.0 \pm 0.26$	4.3	35.8
No. 3	410	7-31	$18.9 \pm 0.25$	5.1	27.0
No. 4	406	15-43	$25.0 \pm 0.34$	6.8	27.2

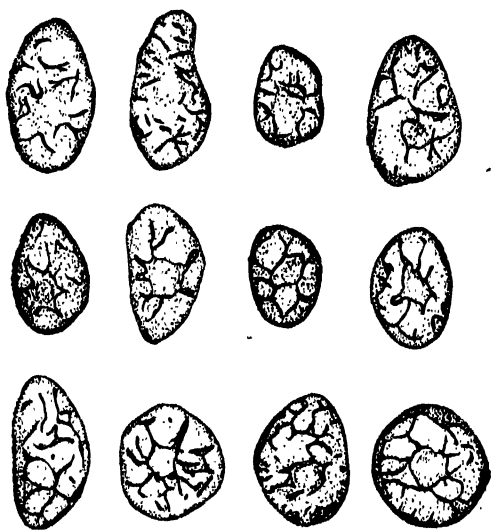


Fig. 13. Silver impregnation of the red cells of *Arca*. ca. 1,000 $\times$ .

As is seen in Table 1, the means of granule-numbers of four individuals fluctuate from 10.4 to 25.0. Besides these four animals there are many individuals in whose red blood cells we may find so numerous granules that the exact counting of them is not possible under the microscope. What is the cause of such extensive fluctuation? To this question I have no answer at present. Here I recall, however, the brown granules of *Urechis*. As I cited above, BAUMBERGER

and MICHAELIS revealed that the older an animal grows, the more numerous the granules become. In the present specimens, the corpuscles which contain less granules were found in the relatively small individuals and vice versa. I regret, that I was not able to make a further statistic investigation of this point, for the collecting season was closed in the course of experiment. The reticulation pattern is demonstrable by the method of TOMITA and others in the red cells which contain no brown granules (Fig. 13).

*Glycimeris vestitus.*

The red corpuscles of *Glycimeris* are similar to those of *Arca* in appearance. They are relatively small and either circular or oval in outline. When viewed on edge they are seen to be very thin, slightly bi-convex discs. The haemoglobin is uniformly distributed throughout the cytoplasm and the nucleus can rarely be distinguished in fresh cells. The numerous yellow-brown granules of irregular size and shape which show varying degrees of Brownian activity are only visible cellular inclusions in the unstained preparations. These granules are positive to the supravital staining with Nile-blue sulphate, neutral red, brilliant cresyl blue etc., but negative to the staining with Sudan III and Scharlach R. The reticulation pattern of the cytoplasm is demonstrable with the method of silver impregnation (Fig. 14).

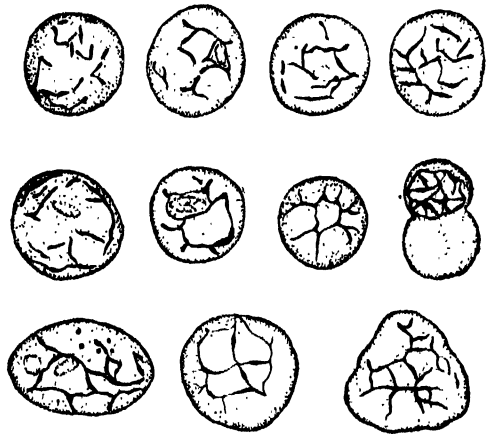


Fig. 14. Silver impregnation of the red cells of *Glycimeris*. ca. 1,000 $\times$ .

Most remarkable feature of these red cells is the polynucleism (Fig. 15). The binucleated red cells are encountered fairly often in the blood of *Physcosoma*, *Lingula* etc., but those red cells which possess three or more nuclei are found very infrequently. In the present specimens, however, the binucleism is very common; tri- or tetranucleited ones are met with frequently, and even penta- or hexanucleism are found upon rare occasions.



TABLE  
*Various properties of the red*

Animals	Shape and size	Nucleus	Neutral red body	Induced granules	Mito-chondria
<i>Urechis</i>	Spherical and biconvex disk. 10-36 $\mu$ in diameter.	Single, small and eccentric. Nucleolus is common.	1-3	The appearance of these granules needs long exposure to dye.	Numerous fine granules or rods.
<i>Thalassema</i>	Same as the above. Deformation occurs very easily.	Same as above.	3-4	Same as above.	Same as above.
<i>Physcosoma</i>	Spherical, oval, or polygonal. Deformation occurs easily. 8-20 $\mu$ in diameter.	Binucleism is occasionally seen. Nucleolus is not common.	2-6	Induction occurs very quickly.	Same as above.
<i>Terebella</i>	Oval biconvex disk. 8-25 $\times$ 6-14 $\mu$	Single and relatively small nucleus.	1-2	Induction is relatively quick.	Same as above.
<i>Arca</i>	Spherical or oval, slightly biconvex and very thin disk. 18-21 $\mu$ in diameter.	Binucleism is occasionally seen. Nucleolus is not common.	Presence of this body is uncertain.	Same as the case of <i>Urechis</i> .	Same as above.
<i>Glycimeris</i>	Nearly equal as the above.	Polynucleism (2-6 nuclei in a given cell) is frequently seen.	Same as above.	Same as above.	Same as above.
<i>Caudina</i> and <i>Molpadia</i>	Elongated oval or spindle. 6-10 $\times$ 10-36 $\mu$	Usually single nucleus. Nucleolus is common.	1-2 in <i>Caudina</i> , 3-4 in <i>Molpadia</i> .	Relatively rapid induction occurs.	Same as above.

2.

*blood cells of eight animals.*

Argentophilic substance (except the preexistent granules)	Oxydase-granules (except the preexistent granules)	Blood pigments	Intracellular crystallization of the blood pigment	Cytoplasmic inclusions (See also Table 3)
Numerous fine granules or short rods.	Spherical granules not so numerous.	Haemoglobin and Haematin.	Rhombic prisms, usually in radial arrangement.	Granules and vacuoles.
Same as above.	Same as above.	Same as above.	Crystallization did not occur.	Same as above.
Typical reticular structure.	Same as above.	Haemerythrin.	Same as above.	Granules, vacuoles and crystals.
Same as the case of <i>Urechis</i> .	Same as above.	Haemoglobin.	Same as above.	Granules.
Typical reticular pattern.	Same as above.	Haemoglobin and Haematin(?)	Rhombic prisms, usually in parallel or radial arrangement.	Granules.
Same as above.	Same as above.	Haemoglobin.	Crystallization did not occur.	Granules.
Same as above.	Same as above.	Haemoglobin.	Same as above.	Granules.

TABLE 3.  
*Comparison of various properties of the preexistent granules in the red blood corpuscles.*

Animals	Colour	Size and Number	Affinity to dyes		Oxydase	Sudan III	Osmic acid	Iron	Silver impregna- tion	Brownian movement
			Supravital staining	After fixation						
<i>Urechis</i> and <i>Thalassoma</i>	Colourless, highly refractive. Occasional granules are brown.	Very numerous. Nearly uniform in size in a given cell.	Intensely positive to Nile-blue sulphate, and neutral red etc.	Basophilic	Positive.	Positive.	Blackens.	Colourless granules are negative, but brown granules are positive to Berlin-blue reaction.	Intensely positive.	Almost negative.
<i>Physcosoma</i>	Colourless or slightly yellowish.	2-4 coarse granules and numerous fine granules.	Same as above.	Basophilic.	Positive.	Negative.	Slightly blackens.	Negative.	Positive.	Active.
<i>Terebella</i>	Same as above.	Usually 2 coarse granules.	Same as above.	Basophilic.	Positive.	Negative.	Same as above.	Negative.	Positive.	Active.
<i>Ara</i>	Yellowish or brown.	They show a good deal of fluctuation in size and number.	Positive to Nile-blue sulphate, neutral red, Janus green B, etc.	Slightly basophilic.	Positive.	Negative.	Same as above.	Positive.	Intensely positive.	Active.
<i>Glycimeris</i>	Yellowish.	Several, small granules.	Same as above.	Same as above.	Positive.	Negative.	Same as above.	Negative.	Positive.	Active.
<i>Candina</i> and <i>Molpadia</i>	Brown.	Usually single, occasionally 2 or more coarse granules.	Intensely positive to brilliant cresyl blue, neutral red, Janus green B, etc.	Same as above.	Positive.	Negative.	Same as above.	Negative.	Slightly positive.	Almost negative.

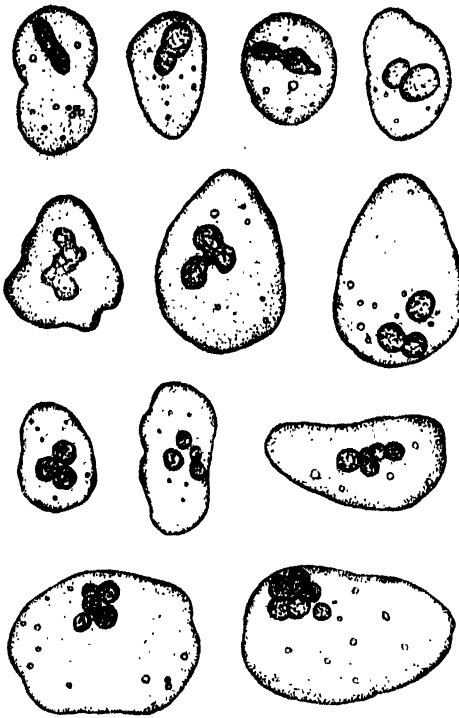


Fig. 15. Fresh and unstained red cells of *Glycimeris* showing the polynucleism. ca. 1,100 $\times$ .

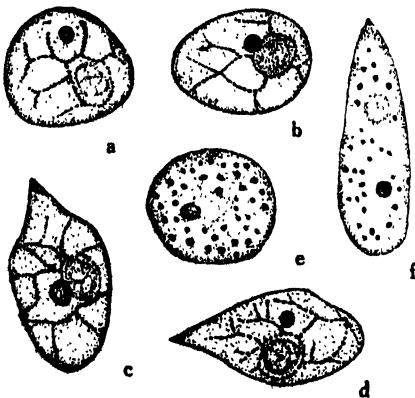


Fig. 16. Silver impregnation of the red cells of *Caudina*. ca 1,200 $\times$ .  
a-d. The normal red cells.  
e, f. The red cells immersed previously in a hypotonic solution.

The nucleus multiplies with amitotic division, and there is seen the polymorphism of nucleus in the course of division.

*Caudina chilensis* and  
*Molpadia roretzii*.

On the properties of red blood cells of these animals I made some informations in my foregoing paper (1936). I described in that paper on the result of silver impregnation of red cells following FUJITA's method, and it was promised that the result obtained by adopting the new method of TOMITA and others will be reported on another occasion. By the silver impregnation using

FUJITA's method, as I stated and figured in the foregoing paper, the argentophilic substance of the normal red cells appeared in the form of numerous and round granules which are arranged in straight or spiral threads resembling beads in appearance. In the red cells which have been previously immersed in a hypotonic sea-water the silver image of red cells appeared also as coarse and not numerous granules, but they were scattered irregularly in the cytoplasm and did not form the shape of beads.

In the present investigation in which new method of TOMITA and others was employed a typical reticular substance appeared (Fig. 16, a-d), as was seen also in the red blood cells of *Physcosoma* and others. Preexistent brown granules are also more or less blackened in this experiment. The reticular structure becomes atypical or is disintegrated into irregularly scattered fine granules by immersing the red cells in a hypotonic salt solution (0.6% NaCl) for about an hour or two (Fig. 16, e, f). The results stated above are summarized in Tables 2 and 3.

## Part II. Leucocytes.

### *Urechis unicinctus* and *Thalassema gogoshimense*.

There is no essential difference between the leucocytes of *Urechis* and those of *Thalassema*, so I will lump here the leucocytes of these animals together under the same title.

In the coelomic fluid of these animals, it is possible to find the four kinds of amoebocytes; 1) hyaline amoebocytes, 2) finely granular amoebocytes, 3) coarsely granular amoebocytes, and 4) compartmental amoebocytes.

1) Hyaline amoebocytes. These cells are relatively infrequent in body fluid of *Urechis* while they are present in a remarkable number in that of *Thalassema*. They show a good deal of variation in size and measure 8-16  $\mu$  in diameter when they take a round shape. The cytoplasm is usually perfectly homogeneous, but one or several yellowish green granules or colourless vacuoles are found in it occasionally. These cells are actively amoeboid, phagocytic, and show an intensive reaction to the vital and supravital staining and oxydase reaction. They have usually a small, round or oval nucleus but occasional cells possess the nucleus which is oval or bean-like in shape, and occupies about a half or more of the total cell volume. Bi- or trinucleated cells are encountered occasionally. The nucleolus is usually found in both types of nuclei. The cytoplasm is intensively basophilic.

2) Finely granular amoebocytes. These are met with infrequently in the body fluid, and measure 10-16  $\mu$  in diameter. The endoplasm is densely filled with acidophilic granules which are nearly uniform in size and somewhat less than one  $\mu$  in diameter. Sometimes the nucleus is entirely covered with granules and is hidden from sight. The cells are amoeboid, and positive to vital staining and oxydase reaction.

3) Coarsely granular amoebocytes, 8-18  $\mu$  in diameter. The colourless

granules which vary in size ( $1-4 \mu$ ) and number are found in the cytoplasm. Besides these, from one to several brownish yellow granules are met with occasionally (Fig. 17). The nucleus is round or oval, located centrally, and possesses usually a single nucleolus. The colourless granules are basophilic and metachromatic. Some of them exhibit also the positive reaction to vitamine A test. Yellow granules are blackened with osmic acid and stained intensely orange red in colour with Sudan III while the colourless granules are negative to these reactions. Moreover these yellow granules are soluble in alcohol, ether, chloroform, benzol and so forth. They are also rapidly distintegrated with alkali, but they resist to acids remarkably. By the application of dilute sulphuric acid, the shade of these granules turns to green. From these properties it is evident that the yellow granules are composed chiefly of a lipochrome.

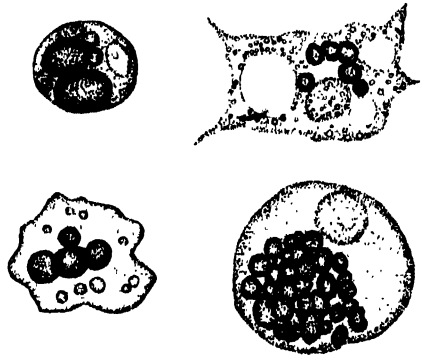


Fig. 17. Coarsely granular amoebocytes of *Urechis*. ca. 1,200 $\times$ .

4) Compartmental amoebocytes. These cells are quite similar to the compartmental amoeboid cells described for ascidian blood in my foregoing paper (1936a), and are seldom found in the blood of *Urechis* alone. Most of these cells do not contain the visible granules or rods in the vacuoles; but some do. The vacuolar contents take usually the vital dyes.

### *Physcosoma scolops.*

The most detailed classification of leucocytes of *Sipunculoidea* was made by Kiyono. He distinguished the leucocytes as follows: 1. Colourless cells of a large or median size; i) large ovoid cells, ii) acidophilic granular cells, iii) basophilic granular cells; 2. Colourless cells of a small size; 3. Cells of a smallest size, i) cells of a relatively large size, ii) cells of a relatively small size. In the present investigation the distinction between cells of type 2 and 3 was not evident, and granular structure of these cells was inconstant, so I recognized them as a kind of leucocytes and called them hyaline amoebocytes. Since the large ovoid cells contain numerous highly refractive and eosinophilic granules, as Kiyono described

already, it seems to be preferable to call them as large acidophilic granulocytes. So I classified the leucocytes of the present instance as follows: 1) hyaline amoebocytes, 2) small acidophilic granulocytes, 3) large acidophilic granulocytes, and 4) basophilic granulocytes. Besides these so-called "urns" were also found in the body fluid almost constantly.

1) Hyaline amoebocytes, 6–14  $\mu$  in diameter (Fig. 18). The cytoplasm is usually hyaline but occasionally several azurophilic granules and fat

globules are found. With Janus green B, the mitochondrial structure is supravitality demonstrable in the perinuclear zone (Fig. 18, k-m). Concerning the structure and vital properties, these cells are very much similar to the corresponding cells of *Urechis* and *Thalassema*.

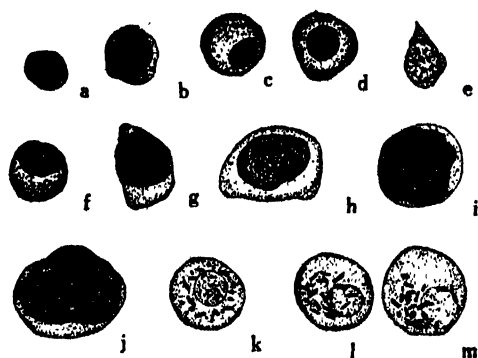


Fig. 18. Hyaline amoebocytes of *Physcosoma*. ca. 900 $\times$ .

a-j. Cells fixed and stained by GIEMSA's method.

k-m. Fresh cells stained supravitality with Janus green B.

In the dry-fixed smears stained by GIEMSA's method, the cells possess usually intensive basophilic cytoplasm, but occasional cells show the polychromatic

staining in various gradations, suggesting the accumulation of haemerythrin in their cell-bodies. A cart-wheel like arrangement of chromatin is also seen in these polychromatic amoebocytes.

2) Acidophilic granulocytes, 10–16  $\mu$  in diameter. These are abundant cells next to red blood corpuscles. The endoplasm is filled with small, clear, spherical or rod-like granules which are eosinophilic in staining reaction (Fig. 19, a-f). The ratio between spherical and rod-like granules in a given cell shows a good deal of variation. KRYONO (l.c.) stated on the acidophilic granulocytes of *Sipunculus* that the spherical granules are original and young, and these turn to short rods with the advance of the cell maturation. Occasional and perhaps young cells possess only a few spherical granules in the perinuclear zone. The granules are stainable supravitality with neutral red, brilliant cresyl blue, Janus green B, Nile-blue sulphate etc., and are positive to Sudan III and Scharlach R. Besides these the yellow or brown granules which are somewhat larger in size

but less in number than the colourless granules are found upon rare occasions. These granules are stainable with Sudan III. The nucleus is relatively large in size, round or oval in shape, and coarsely granular in internal structure. It is centrally situated and is obscured with the granules frequently. A few blunt pseudopodia may be present peripherally, and the cell is continually changing its shape as it moves through the field of the microscope. The vital granules of various dyes are round in shape, remarkably great in number and chiefly located in the perinuclear zone. The oxydase granules are similar to the vital granules in general, but more or less smaller and fewer than the latter.

3) Large acidophilic granulocytes. These cells are one of the constant elements in the body fluid of *Physcosoma* and are very similar to the lamprocytes of earth-worms in size and appearance (Fig. 19, n s). They are usually large ovoid disks, measuring  $16-38\mu$  in longer,  $12-30\mu$  in shorter diameter and  $5-8\mu$  in thickness. Occasional cells form a twin by uniting with another cell. In this case the longer diameter becomes, of course, greater than the above. CUÉNOT (1891) described on the "vesicules énigmatiques" in the blood of *Sipunculus* and *Phascolosoma*. These corpuscles are similar to the present cells in appearance, but the former far exceed the latter in their size, measuring  $44-360\mu$  in diameter. The endoplasm is compactly filled with highly refractive round granules which vary from  $1.5$  to  $4\mu$  in diameter but are nearly of the same size in a given cell. These granules are stained intensely with neutral red, Janus green B, Nile-blue sulphate, etc. They are dissolved, however, gradually and become invisible by the staining with brilliant cresyl blue. With osmic acid they are blackened slightly, and are positive to the staining with Sudan III or Scharlach R. Very fine granules which are blackened intensely with osmic acid, positive to Sudan III staining and oxydase reaction appear in the cytoplasm among the large granules. By GIEMSA's staining method, these large granules are stained pink in colour, showing the eosinophilic reaction. The granules are dissolved frequently by an unsuitable procedure of fixation. In these cases the cytoplasm remains as an alveolar structure which is basophilic in staining reaction (Fig. 19, s).

These corpuscles are usually binucleated. This fact has not been described in other *Sipunculoidea*, so far as I am aware. One of the nuclei is situated constantly in the peripheral cytoplasm which is slightly extruded to accept it. Owing to the pressure of granular cytoplasm the nucleus is more or less flattened, but no sign of degeneration is visible. Another



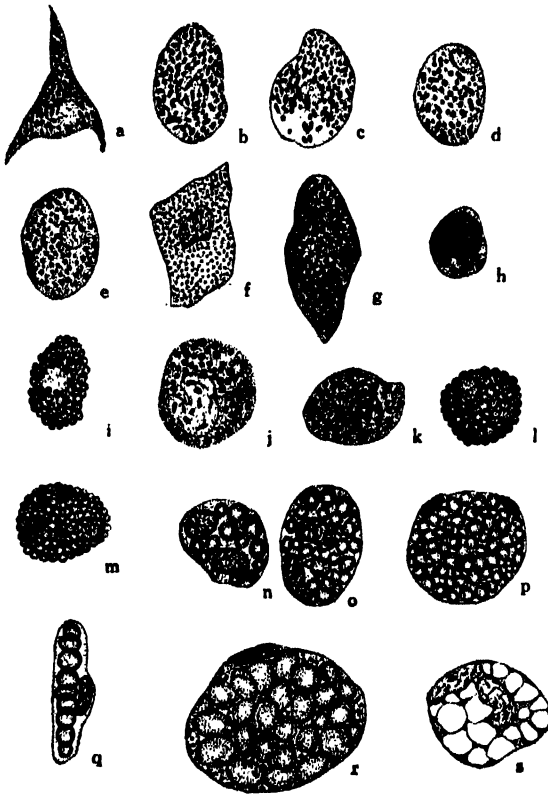


Fig. 19. Granulocytes of *Physcosoma*. ca. 1,000 $\times$ .

a-g. Fresh and unstained acidophils.

h-m. Fresh and unstained basophils.

n-s. Large acidophils in which q, fresh cell seen on edge; s, fixed and stained cell by GIEMSA's method.

ance and chromatic property. Namely they are usually spherical, basophilic, nearly uniform in size in a given cell and less numerous than the acidophilic granules (Fig. 19, h-m). The nucleus is usually oval or bean-shaped, and rich in chromatin. The vital properties are nearly similar to those of small acidophilic granulocytes.

5) Urns. One of characteristic elements of coelomic fluid of *Sipunculoidea* is so-called "urn" or "ciliated corpuscle". The urns were discovered by KROHR (quoted from CUÉNOT), and the detailed investigations were made by CUÉNOT (l.c.), SELENSKY (1908) and others. Urns of *Physcosoma* consist of several or a dozen of cells and measure 15-40  $\mu$

nucleus is centrally or peripherally situated, oval or round in shape and somewhat larger than the former. The chromatin granules of both nuclei are fine and numerous.

The surface of cells is bound with a membrane which is clearly discernible under the microscope. The amoeboid movement and phagocytosis were not detectable in any case. A few and small vital granules of neutral red and others are found in the cytoplasm among the preexisting granules.

4) Basophilic granulocytes. These are infrequent cells of the same size as the small acidophilic granulocytes. The granules found in the endoplasm are all of one kind in appearance

in diameter (Fig. 20). As SELENSKY described, cupola, disk and neck are also distinguishable in the present specimens. Under the microscopic observation the urns swim very actively to and fro in the body fluid. Suspending red corpuscles are stirred up and thrust aside by the ciliary

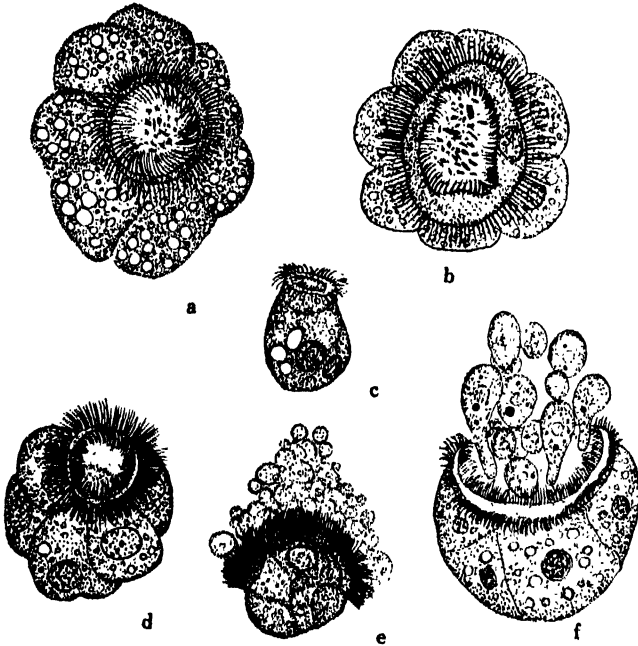


Fig. 20. Urns of *Physcosoma*. ca. 800 $\times$ .

movement. Degenerating blood corpuscles and various formed substances, which are previously introduced intraperitoneally such as oil droplets, India ink, pigment particles etc., are gathered at the mouth part of urns and agglutinated to an irregularly shaped conglomeration (Fig. 20, e, f).

The cells of urns are filled with numerous globules which vary in size and are stained intensely with Sudan III, Nile-blue sulphate, neutral red and so forth. With Janus green B, fine and numerous granules which may be identified as the mitochondria are demonstrable. The oxydase reaction is intensely positive in all the cells of urns.

*Terebella* sp. (*T. debelis*?)

In contrast with the leucocytes of *Physcosoma*, those of the present animals show differentiation of a lower degree. Most leucocytes are

spindle-shaped amoebocytes, measuring about  $14-30\ \mu$  in larger, and  $2-6\ \mu$  in shorter diameter (Fig. 21, a-d). These are found in the ratio of about

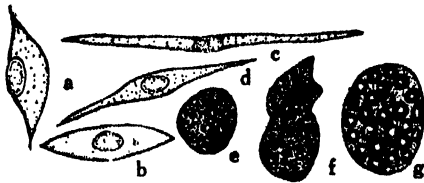


Fig. 21. Leucocytes of *Terebella*. ca.  $1,000\times$ .

a-d. Hyaline amoebocytes.

e-g. Granular amoebocytes.

$1:100$  to erythrocytes. They deform, however, their shape in the blood which is standing still, and begin the amoeboid movement, protruding a few blunt pseudopodia. The young type of these leucocytes possesses perfectly hyaline cytoplasm and a relatively large nucleus which is rich in chromatin. With the

growth of the cells numerous very fine and basophilic grauules become visible in the endoplasm. A single brown granule is found also upon rare occasions.

Another kind of leucocytes is the granulocytes which are encountered upon rare occasions. They are nearly of the same size as the erythrocytes. In the living condition the cytoplasm is filled with small, clear spherical granules (Fig. 21, e-g). These granules are usually of the even size in a given cell, and amphophilic with more or less inclination of basophilia in staining reaction. The nucleus is spherical, coarsely granular, and usually obscured with granules.

#### *Arca inflata*.

In the blood of *Arca*, SATO (l.c.) described a kind of leucocytes, measuring  $8\ \mu$  in average diameter. They are amoeboid, and possess a small, round nucleus and fine refractive granules. Besides these leucocytes, the granulocytes were found occasionally in the present specimens.

1) Leucocytes or hyaline amoebocytes. In this group two kinds of cells may be distinguishable at least — small and large lymphocytes. The former (Fig. 22, a-c) is  $4-6\ \mu$  in diameter, its nucleus is large or small, and the cytoplasm is intensely basophilic. There is no granular substance in the cytoplasm of this cell. The latter (Fig. 22, d-j) is  $8-12\ \mu$  in diameter. Great majority of them are cells with small and round nucleus. These are abundant next to erythrocytes and fully described by SATO. Besides these, large cells with a large nucleus are encountered infrequently and it is supposed that these may be ancestral cells of the small nucleated leucocytes or erythrocytes. The nucleus is ovoid, bean or kidney in

shape, and occupies the greater part of the total cell-volume. The chromatin is coarsely granular and shows the cart wheel-like arrangement, occasionally. The cytoplasm is stained gray-blue by GIEMSA's method, and

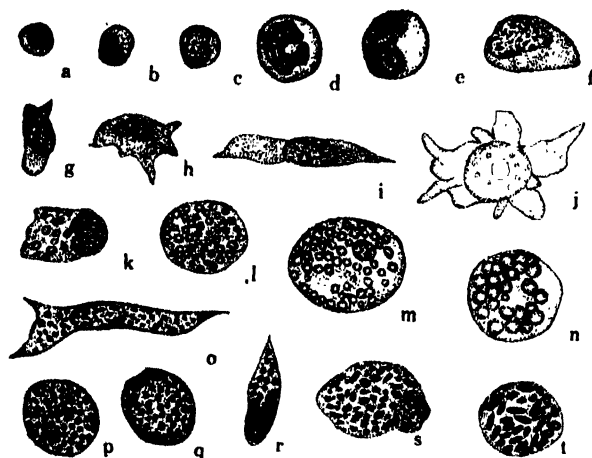


Fig. 22. Leucocytes of *Arca*, ca. 1,000X.

a-j. Hyaline amoebocytes. k-t. Granular amoebocytes.

a few round, basophilic or azurophilic granules are found in it upon rare occasions. Polychromatophilic cytoplasm is seen in the occasional cells of this group.

Hyaline amoebocytes are actively amoeboid and phagocytic. They show positive reactions to the vital staining and indophenol blue synthesis.

2) Granular amoebocytes. These are also an infrequent element in the blood of *Arca*. Great majority of granulocytes of the present specimens are somewhat smaller than the corresponding cells of *Physcosoma*. The granules are usually spherical or ovoid and of the uniform size in a given cell (Fig. 22, h-t). The nucleus is round or oval and frequently hidden with the granules. In smears stained with GIEMSA's method the granules show the amphophilic reaction with an inclination to basophilia.

The granulocytes are amoeboid and phagocytic, but less active than the hyaline amoebocytes. Vital staining and indophenol blue synthesis are also positive remarkably.

#### *Glycimeris vestitus*.

The usual kinds of leucocytes such as hyaline amoebocytes, finely granular amoebocytes and coarsely granular amoebocytes are encountered

also in the blood of the present specimens.

*Caudina chilensis* and *Molpadia roretzii*.

KAWAMOTO (1928) reported the leucocytes of *Caudina* in detail. The present author made also some informations on this subject in the previous papers (1933, 1936). Of the fusiform corpuscles it is accepted that they possess no nucleus. But I found many of nucleated ones in the fixed smears of the blood of *Caudina*, stained by the method of GIEMSA (Fig.

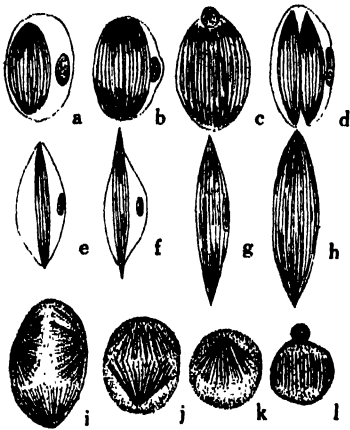


Fig. 23. Various forms of fusiform corpuscles in the blood of *Caudina*. ca. 1,000 $\times$ .

23, a-g). The fibres of nucleated fusiform corpuscles are in a majority of cases arranged in a single bundle, but those were seen occasionally in which two, three or several bundles run in various directions (Fig. 23, i). Such ones were seen also in which the end of fibres stand widely apart. Moreover there exist those fusiform corpuscles in which no striation is visible. The latter appear to be formed in the blood corpuscles. They are small, oval, hyaline and refractive at first, but they grow larger and taper by and by, and the longitudinal striations become visible. These changes are very similar to the crystal formation in the red

blood corpuscles.

Haematopoiesis of Gephyreans.

It is generally accepted by many authors that the coelomic corpuscles of invertebrates are formed by the proliferation of endothelial cells and the mitoses of cells in the circulating fluid. An evident was seen indicating that probably the parietal and visceral peritonea of the present specimens are also an essential locus of haematopoiesis. SPENGLER (1879), JAMESON (1899), IKEDA (1904) and others studied on the histology of various Gephyreans. JAMESON, for example, stated that the peritoneum of these animals (*Thalassema neputui*) is composed of lenticular cells of somewhat polygonal outline in section of surface. In the present specimens similar relation was observed. The peritoneal cells are intensely

positive to vital, supravital staining and oxydase reaction. For the study of haematopoiesis, worms fixed with HELLY's fluid were sectioned and stained with GIEMSA's stain or DELAFIELD's haematoxylin and eosin. In several places of peritoneum it is possible to see that the cells, first hypertrophied and elongated intensely, projecting into the lumen of coelom (Fig. 24). The mitotic division may occur at this stage. Accordingly the peritoneum assumes an appearance of pseudostratified epithelium. Proliferation of hypertrophied cells from peritoneum is followed by the formation of a hemoblast. The nuclear volume is usually small in the cells of advanced metamorphosis, while it amounts to about a half or more of the total cell volume in the young cells, but there are exceptions to such generalized principle. The cytoplasm is at first hyaline and deeply basophilic but it becomes polychromatophilic or eosinophilic in occasional cells, showing the differentiation to the direction of erythrocytes. The presence of nucleolus is frequently seen in the nucleus of these cells. The granulation of cytoplasm occurs also in this locus.

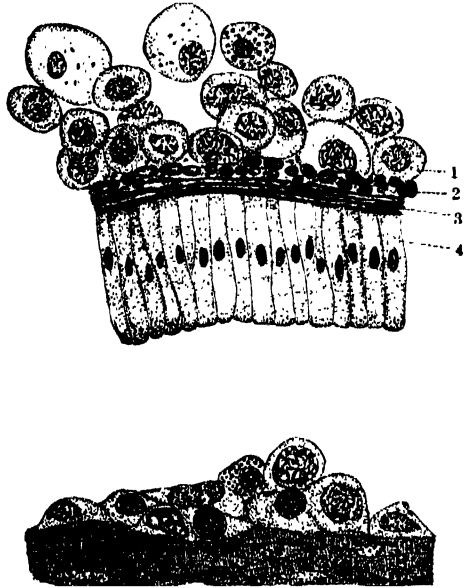


Fig. 24. Hypertrophied and proliferating splanchnic (a) and somatic (b) peritoneum of *Physcosoma*. ca. 800 $\times$ .

1. Peritoneal cells. 2. Longitudinal muscles. 3. Circular muscles. 4. Epithelium of intestine.

#### DISCUSSION

The results stated in Part I are summarized in Tables 2 and 3.

It is generally accepted in recently published descriptions (ROMIEU 1923, DAWSON 1932. and others) that red coloured corpuscles of invertebrates are usually nucleated but not amoeboid. In the present investigation the red blood cells of *Thalassema*, *Caudina* and *Molpadia* possessed conspicuous protrusions respectively, when they were exposed to an

unfavourable condition, but the amoeboid movement was not seen in any case.

In the eight species here described all the red corpuscles are circular or oval in outline and have disk-like form. Their sizes show a good deal of variation even within a species or an individual, contrary to the erythrocytes of vertebrates.

As it was pointed out by DAWSON (1932b) granular inclusions appear to be characteristic of almost all invertebrate-erythrocytes. In the present case all species of animals possessed the red cells in which coloured or colourless granules in variable number, shade and shape are contained. The granules of red cells of *Urechis* and *Thalassema* show the characteristics of fat most intensely. Moreover, even the presence of vitamin A in the granules is also detectable. These facts suggest that the red cells of these animals serve also the purpose of transportation or reservoir of nutrient substances, besides the respiratory function. In other words, they still remain in a lower stage of differentiation from the leucocytes. As is seen in Table 3, the granules of other six species are also blackened with osmic acid, suggesting the existence of fatty substances in them. The granules are extensively positive to the oxydase reaction with no exception. As it was pointed out by KATSUNUMA (1919), KIYONO (1927) and others, almost all leucocytes of invertebrates possess the oxydase-granules. In the vertebrates, however, the appearance of these granules is limited to the leucocytes of myeloid origin. In the present case that the oxydase granules appeared not only in leucocytes but also in red cells suggests also the low differentiation of red cells of invertebrates. As was frequently stated in text-books, that among invertebrates there are no cells which are exactly comparable with the erythrocytes of the vertebrates is the truth, so far as the facts are concerned with these points. There are, however, some facts which contrast with this opinion. It is evident from the present observation that patterns resembling the segregation apparatus and the reticulation of vertebrate-erythrocytes can be obtained in the red cell of the present specimens, by the application of vital dyes or silver nitrate. The segregation apparatus or neutral red bodies of vertebrates are studied extensively by JORDAN (1925), DAWSON (1928, 1929a, 1932a, 1933), YASUZUMI and others (1934) etc. DAWSON (1932a) concluded that the number of so-called segregation apparatus is nearly constant within a given species of vertebrates. This was true also in the present investigation, as was seen in the red cells of *Urechis*, *Thalassema*, *Caudina* and *Molpadia*. ROMIEU (1923) obtained a coarse-

meshed network in the corpuscles of *Glycera tessellata* after haemolysis and suggested that it might correspond to the substantia granulo-filamentosa (reticulation substance) of the vertebrate-erythrocytes. DAWSON (1932b) described also patterns resembling the vertebrate reticulation in the red cells of four invertebrates, *Phascolosoma gouldi*, *Glycera dibranchiata*, *Thyone fusus* and *Arca transversa*. The results of the present observations accord well with their descriptions.

The most clear and typical pattern of reticulation was obtained, however, in the red corpuscles of *Physcosoma*, *Arca*, *Glycimeris*, *Caudina*, and *Molpadia*, using the silver impregnation method of TOMITA and others. As was mentioned above, TOMITA, KAWABE and YUBA (1934) made systematic studies on the minute structures of erythrocytes of different classes of vertebrates with a new method of silver impregnation, and distinguished the fine cellular structures as follow: A. Reticular type; 1. Fundamental type, 2. Transitional type, 3. Simple type; B. Negative type; C. Specific type; 1. Fibrillar type, 2. Type of droplet, 3. Type of filament. Since the typical pattern of fundamental type is found in fish and amphibia it was called also "amphibian type". In a similar manner, the simple type was named "avian type" and the negative type "mammalian type". By the same method, during the earlier development of chick embryos, the silver impregnated structure of erythrocytes shows the amphibian type and with further development the structure approaches to avian type. A similar result was obtained also in the embryo of rabbits. These are regarded as an evidence of the biogenetic law.

It is of interest to find what kind of silver-impregnated structure exists in the erythrocytes of invertebrates. In the eight species here described the red cells of *Physcosoma*, *Arca*, *Glycimeris*, *Caudina* and *Molpadia* showed a typical pattern of amphibian type, and the red cells of the rest were of the negative type. That there is no pattern peculiar to invertebrate erythrocytes is evident, so far as the animals of present investigation are concerned. KAWABE (1934) and IZUMI (1935) made observations on the effects of various reagents on the fine structure of vertebrate-erythrocytes. Their results are in accordance with each other on a point that the fine structure is deformed or disintegrated by the action of various reagents. A similar result was also obtained in the present investigation.

From these facts mentioned just above it is concluded that the erythrocytes of some invertebrates bear a close resemblance in their fine structure to those of the vertebrates, especially fishes and amphibia. Why did the erythrocytes of *Unachis*, *Thalassema*, *Terebella* and full grown *Arca* show



no reticulation pattern by the silver-impregnation? As to this question I know nothing at present. In the present investigation, however, it seems be possible to conclude that there are numerous preexistent granules which are intensely positive to silver impregnation throughout the erythrocytes of negative type. Moreover, the secondary fine granules of supravital staining are also difficult to appear in the cells of the negative type. I suppose, therefore, that there exists some relation between the preexistent granules and the cellular fine structure. Further investigation is necessary on this point.

The red blood cells of present investigation are prepared with a definite cell membrane. They change their shape when they go through a narrow space, but they return to the normal one as soon as they passed there. The red cells are invaded with saponin, and the main part of the cell body flow out through a localized opening. They swell like a balloon when immersed in a hypotonic solution while they assume a thorn-apple shape in a hypertonic solution. These are also similar to the erythrocytes of vertebrates.

The nucleus of matured erythrocytes is usually small und round. In the young type of cells, however, a large nucleus in which chromatin granules are arranged in a shape similar to that of the normoblast of vertebrates is seen not infrequently. The red cells of some invertebrates (*Glycimeris*, *Physcosoma*, *Arca* etc.) show the polynucleism in a considerable frequency while those of the vertebrates contain usually a single nucleus. On the contrary the polynucleism of white corpuscles, which is exceedingly common in the blood of the vertebrates, was not found in these invertebrates. It seems to me that the blood corpuscles of both groups of animals show a contrast in respect of the polynucleism.

From the data mentioned above a conclusion may be drawn that the erythrocytes of some invertebrates possess the same characteristics of vertebrates in general, except the constant existence of various cytoplasmic inclusion.

Concerning the facts stated in Part II there is little to be discussed here. Great majority of leucocytes, as were mentioned above, are positive to the reactions of indophenol blue synthesis and vital staining, showing the low grade of differentiation compared with those of the vertebrates. Polymorphism or lobulation of nucleus of granulocytes is very common in the vertebrates, but it was not found in any specimens of the present case, as was mentioned above. The chromatin granules, however, assume frequently certain arrangement which makes one recall the lobulation of

nucleus to the mind. Namely they are more densely arranged in the peripheral zone of a nucleus than in the central part of it. Such a nucleus takes the appearance of a ring or, more frequently, an unclosed ring which leads investigators to misjudgement, recognizing it as a lobulation of nucleus at an early stage. Bean- or kidney-shaped nucleus which is seen in the transitional form of vertebrate's lymphocytes, however, is found also frequently in the hyaline amobocytes of the present specimens.

The granules of leucocytes are similar to those of vertebrates in appearance. But most of them are acidophilic or amphophilic with more or less indication of acidophilic, and pure basophilic granulocytes are encountered rather infrequently, contrast with the case of vertebrates. That fatty granules or globules exist abundantly is also a characteristic of the invertebrates-leucocytes.

#### SUMMARY

1. The formed elements in the body fluid of *Urechis unicinctus*, *Thalassema gogoshimense*, *Physcosoma scolops*, *Terebella* sp., *Arca inflata*, *Glycimeris vestitus*, *Caudina chilensis*, and *Molpadia roretzii*, were studied.

2. The properties of red cells and the granular inclusions are summarized in Tables 2 and 3. From the data found in these tables it may be concluded that the red cells of these animals are very rich in granular inclusions, but they possess the cytoplasmic structure similar to that of vertebrate-erythrocytes.

3. The ordinary kinds of amoebocytes — hyaline and granular amoebocytes — were distinguishable in all these animals. In the coelomic fluid of *Physcosoma* large granulocytes, similar to the lamphocytes of earth worms, and the urns were seen besides the ordinary formed elements.

4. Haematopoiesis of peritoneal epithelium was seen in *Urechis*, *Thalassema*, and *Physcosoma*.

I wish to take this opportunity to thank Dr. S. HATAI for his interest during the course of this investigation and for revising the manuscript in spite of his precious time. My thanks are also due to Dr. S. TAKATSUKI and Mr. T. KAWAMURA for their kind suggestions on the collection of materials used for the present investigation.

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# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## VIII. SUPPLEMENTARY NOTE ON THE FORMED ELEMENTS IN THE COELOMIC FLUID OF SOME BRACHIOPODA<sup>1)</sup>

By

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(With nine figures)

(Received August 23, 1937)

In the previous paper (1936) I gave some informations on the coelomic fluid of a brachiopod, *Terebratalia coreanica*. Recently I was bestowed an opportunity to get abundant specimens of *Coptothyris grayi* (DAVIDSON) (= *Coptothyris grayi aomoriensis* HAYASAKA), *Lingula unguis* LINNAEUS (= *Lingula anatina* BRUGIÈRE). I wish to describe here about some observations made upon the formed elements in the coelomic fluid of these animals.

### *Coptothyris grayi*

These animals are very abundant in Mutsu Bay, and were collected exclusively in the vicinity of the Asamushi Marine Biological Station.

The following types of cells are distinguishable in the body fluid: 1. hyaline amoebocytes, 2. coarsely granular amoebocytes, 3. finely granular amoebocytes, 4. amoebocytes with orange granules, 5. amoebocytes with brown granules, 6. amoebocytes with red granules, 7. vesicular cells, and 8. spindle bodies or fusiform corpuscles.

Since the cells of the first four kinds are not essentially different in appearance, function and occurrence from those cells of *Terebratalia*, so I will omit their description. As to the amoebocytes with red granules there is also no fundamental difference from those cells of the animals just mentioned above, except the point of the number of red granules. As I described in the previous paper, the number of these granules of *Terebratalia* was from one to six in a cell, although there were found occasionally those cells in which a dozen or more of granules were conglomerated like a mulberry-formed packet. In the present instances,

<sup>1)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 143.

however, one or two dozens are the usual number of red granules (Fig. 1, a-c), and it is possible to encounter a cell which contains one hundred or more granules, not so infrequently. There is found a little relation

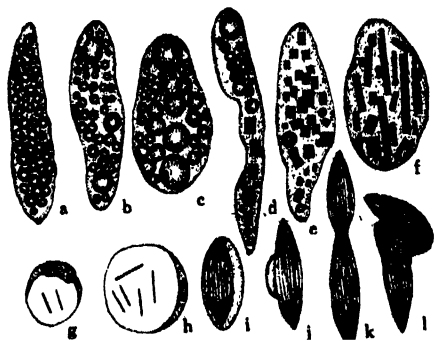


Fig. 1. The coelomic corpuscles of *Coptothyris grayi*. ca. 900 $\times$ .

a-c. Amoebocytes with red granules.

d-f. Amoebocytes with red crystals.

g, h. Vesicular cells.

i-l. Fusiform-corpuscles.

between the number of red granules and the size of shells. The colourless granules which were very common in the red cells of *Terebratalia* are rarely found in the present case, and it may be due, perhaps, to the abundant existence of red granules. The pigment in the red granules possesses a tendency to crystallize within the cells. Under the prolonged microscopic observation the red granules which were at first round or oval in shape become gradually tetragonal or rectangular (Fig. 1, d, e). The granules fuse

together by and by, and the crystals grow larger and larger (Fig. 1, f). Such a cell is frequently seen in which some granules still remain in the natural shape while and others turned already into the crystals. Such intracellular crystallization is not characteristic of the cells of *Coptothyris* only, but is also found in those of *Terebratalia*. Vesicular cells are a rather infrequent element in the coelomic fluid of *Coptothyris*. These are cells measuring about 6-15  $\mu$  in diameter and contain usually a single large vacuole surrounded by a thin layer of cytoplasm which is thicker in the region where the nucleus is located. The vacuole is filled with colourless or slightly pale cinnamon-pink coloured fluid. This fluid takes various dyes such as neutral red, brilliant cresyl blue, Nile-blue sulphate, etc., supravitaly. Several crystals of needle-shape are seen frequently in it (Fig. 1, g, h). Indophenol-blue synthesis occurs in this fluid. By the reexamination of the coelomic fluid of *Terebratalia* this kind of cells was also found in it.

In the foregoing paper I could not state positively as to the presence of spindle bodies in the blood of *Terebratalia*. In the present investigation, however, it was ascertained in the both animals, *Terebratalia* and *Coptothyris*. Spindle bodies of these animals are so sparsely found that

one may examine several cover glass preparations of body fluid without finding one. Since very numerous animals, I am happy to say, were obtained in the present investigation, I brought together the body fluid of several individuals in a test tube; it was centrifuged and the precipitation was examined under the microscope. Only a few spindle bodies were found in an amount of such concentrated body fluid (Fig. 1, i-l). I kept, on the other hand, several individuals in the fresh water to know whether such unfavourable condition causes the increase of spindle bodies or not. There is, however, no evidence of new formation of these enigmatical bodies in this case. Of the spindle body the detailed description will be seen in the next section.

The structure of the cirri of *Coptothyris* was quite similar to that of *Terebratalia*. The presence of endothelium which serves as a transversal septum and divides the lumen of cirrus into two was clearly seen in the present specimens (Fig. 2).

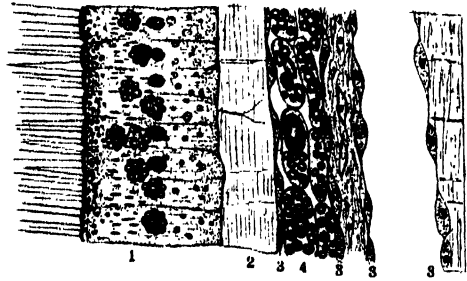


Fig. 2. The optical section of a cirrus. ca. 600 $\times$ . Most of the right half is omitted in this figure.

1. Ciliated epithelium in which granular amoebocytes are seen.
2. Elastic layer.
3. Endothelium.
4. Amoebocytes with red granules.

#### *Lingula unguis* (= *Lingula anatina*).

Regarding the histology of *Lingula unguis* LINNAEUS=*Lingula anatina* BRUGIÈRE there is an excellent work of YATSU (1902), in which he describes also of the formed elements found in the coelomic fluid. He distinguished three kinds of coelomic corpuscles; 1. blood corpuscles, 2. leucocytes, and 3. spindle bodies. I obtained recently many specimens of *Lingula unguis* by the kindness of Mr. K. BABA who collected them in the vicinity of the Amakusa Marine Biological Station. The results of the observation on the coelomic corpuscles of these animals are in accordance with the description of YATSU, save for some details which I am going to put down in the following.

I distinguished five kinds of formed elements in the coelomic fluid; 1. red blood corpuscles, 2. hyaline amoebocytes, 3. eosinophilic granulo-



cytes, 4. basophilic granulocytes, and 5. spindle bodies.

1. Red blood corpuscles. These are 'blood corpuscles' in the term of YATSU. In my previous paper (1936a) I stated incorrectly that 'blood corpuscles' are, perhaps, the cells corresponding to the hyaline amoebocytes of *Terebratalia*. I must express my regret for such misunderstanding of his description. The source of my ridiculous confusion is found in the following description of YATSU: "It ('blood corpuscle') is *colourless*, transparent and —". In my present observation also the blood corpuscles do not appear red but merely of a very pale yellow tinge when they were seen one by one; a distinct eosin-pink colour which resembles that of red cells of *Gephyreans* is, however, seen when a number

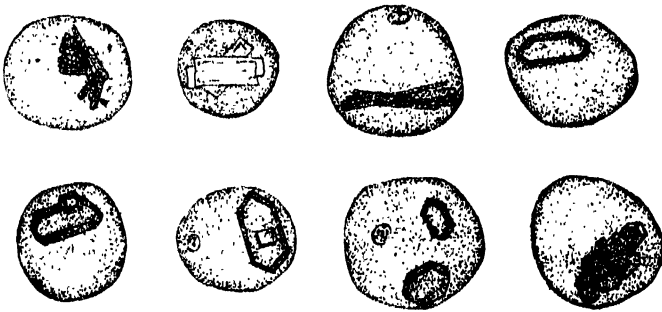


Fig. 3. Red blood cells with haemoglobin-crystals. ca. 1,200 $\times$ .

of 'blood corpuscles' are brought together. That these cells contain a blood pigment in the cytoplasm is also evident from the fact that the intracellular crystallization of the pigment takes place easily in vitro (Fig. 3). As is seen in my last paper (1937)-the same phenomenon was observed in the red cells of *Arca* and *Urechis*, and it was supposed that such crystal formation may be due to the gentle withdrawal of water from the cytoplasm. In the present investigation the intracellular crystals appeared when the cells were exposed to absolute alcohol or the sugar solution of a considerably high concentration. By the actions of these reagents, perhaps, the water of cytoplasm is withdrawn, the blood pigment becomes more concentrate, and consequently the pigment begins to separate out as the crystals in situ. Some cells contain several crystals which are columnar in shape and aggregated like a rosette, but a majority of cells possess one to several rhombic tabular crystals. These crystals are slightly pale cinnamon pink in colour. By the TEICHMANN's test the crystals just aforementioned are demonstrable. I suppose, accordingly, that the blood

pigment of present instance may belong to a haemoglobin.

The red blood corpuscles are, as was already described by YATSU, usually depressed in one or two places in normal conditions (Fig. 4). The nucleus is not seen owing to the presence of blood pigment. Usually one, occasionally two or three, small and refractive granules similar to those which are commonly found in the red blood corpuscles of various invertebrates are visible almost constantly. These granules are stainable supravivally with Cresylechtviolett, brilliant cresyl blue, Nile-blue sulphate, Janus green B, neutral red, Scharlach R. etc. and are positive to the indophenol blue synthesis. Prolonged observation under the microscope causes the depression of red blood corpuscles to disappear and they become a disc with slight biconvexity. The nucleus of the red corpuscles in such condition becomes visible. It is usually round or oval, but occasionally rod, cocoon, U-, V-, or Y-letter like in the shape (Fig. 5). The ordinary cells possess a single nucleus, but those cells which have two or three nuclei are encountered upon rare occasions. A single nucleolus is found in the nucleus. Chromatin substance is fine and numerous, and nuclear appearance, therefore, is rather compact.

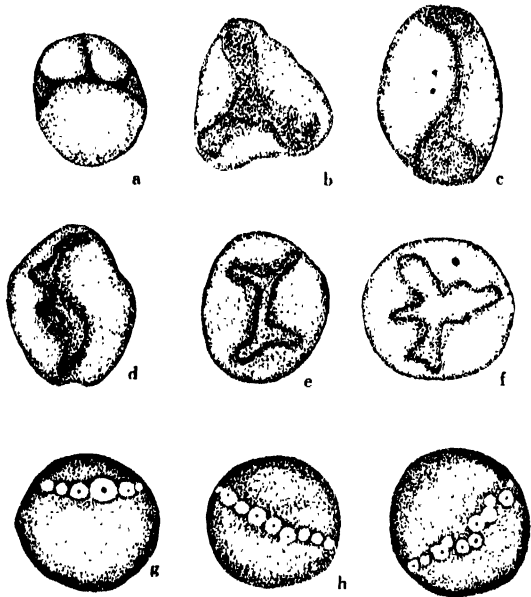


Fig. 4. Red blood cells. ca. 1,200 $\times$ .

a-f. Fresh and unstained corpuscles.

g-i. Fresh corpuscles stained supravivally with brilliant cresyl blue showing clear and regularly arranged vacuoles.

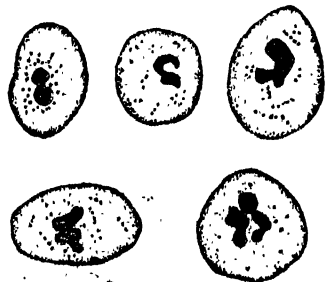


Fig. 5. Red cells stained supravivally with Janus green B showing polymorphic nuclei and mitochondria. ca. 1,000 $\times$ .

Supravital staining with brilliant cresyl blue, Cresylechtviolett, etc. causes the appearance of several vacuoles which form usually a line on the equatorial surface of cells and are stained slightly in pinkish tinge (Fig. 4, g-i). Occasionally a deep blue or purple coloured particle in quivering movement is found in most of the vacuoles. The formation of those vacuoles in the red cells of marine invertebrates was found by DAWSON (*Phascolosoma* 1932) and the present author (*Urechis* and *Thalassema* 1937), but the arrangement of vacuoles was not so regular as in the present instance. Besides the preexisting refractive granules, fine rod or granules in various numbers appear in the cytoplasm by the supravital staining (Fig. 5). These granules or rods nearly fill the cytoplasm of the small (perhaps young) cells, while they found very sparsely or are entirely absent in the large and full grown cells, and there is found every gradation between these extremes (Fig. 6).

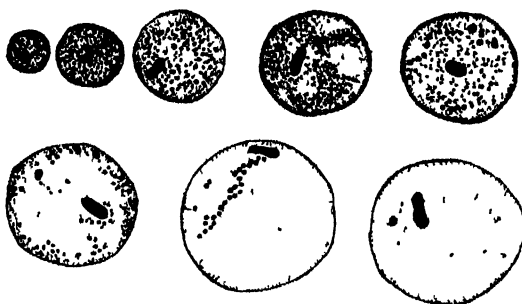


Fig. 6. Various stages of red-cell development. Supravitaly stained with Cresylechtviolett. ca. 1,200 $\times$ .

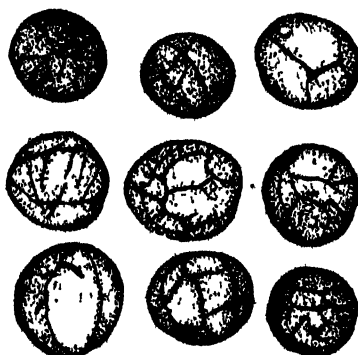


Fig. 7. Silver impregnation of red cells. ca. 1,000 $\times$ .

tion between these extremes (Fig. 6).

The present author (1937) detected the presence of a typical reticular pattern in the red cells of *Physcosoma*, *Arca*, *Glycimeris*, *Caudina* and *Molpadia*, using the method of TOMITA and others. In the present specimens that pattern was also

found almost all red cells (Fig. 7).

In the fixed smears stained by GIEMSA's method the cytoplasm of red cells, shows, of course, the eosinophilic reaction.

2. Hyaline amoebocytes. These cells are one of formed elements rarely met with in the coelomic fluid, measuring 5-12  $\mu$  in diameter (Fig. 8, a-f). They are colourless, hyaline, amoeboid and phagocytic cells similar to the corresponding cells of other animals. In the fixed smears stained

with GIEMSA's stain the cytoplasm shows various tinges from light blue to blue violet, and contains one or several small vacuoles. The nucleus is stained always rosolanc purple. The hyaline amoebocytes are difficult to distinguish from the young erythrocytes with supravital preparation,

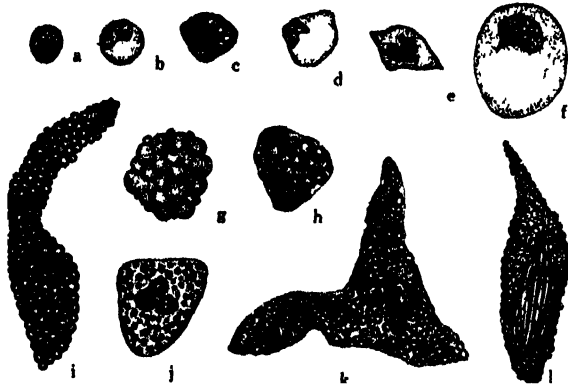


Fig. 8. Leucocytes of *Lingula*, ca. 1,000 $\times$ .

a-f. Hyaline amoebocytes

g-h. Coarsely granular amoebocytes.

i-k. Finely granular amoebocytes.

l. Amoebocytes with an ingested fusiform corpuscle.

and on this basis, I suppose, YATSU would have omitted the former from his description. It is possible, however, to distinguish the young erythrocytes from the hyaline amoebocytes with their polychromatophilic nature to the GIEMSA's stain.

3. Eosinophilic granulocytes. These are corresponding cells of 'leucocytes' in YATSU's report. They are amoeboid, phagocytic and positive to the vital staining, Nadi-reaction, etc. (Fig. 8, i-l). The granules in a given cell are nearly uniform in size (approximately  $0.6-2.0\mu$  in diameter) and show the oxyphilic reaction. The nucleus is concealed with the granules. These cells are next abundant to the red cells, and an agglutinated mass composed of several dozens of these cells are found usually here and there among the red cells by the microscopic observations.

4. Basophilic granulocytes. These cells are very much similar to the eosinophils in size and structure but they possess more coarse granules in comparison with the eosinophils and the granules are stained, of course, metachromatic basophilically by the method of GIEMSA (Fig. 8, g, h). These cells are encountered very rarely in the marginal lacuna.

5. Spindle bodies. A review of the literature on the spindle bodies

of *Lingula* was given by YATSU (1902). Besides *Brachiopoda* these bodies were found in the body fluid of *Bryozoa* (CORI 1891), *Annelida* (MRÁZEK 1910), *Echinoidea* (THÉEL 1920-'21; DAWSON 1932), *Holothuroidea* (KAWAMOTO 1927; OHUYE 1936), *Mollusca* (ORTON 1923), etc., so far as I am aware. It seems to me that the spindle bodies or fusiform corpuscles of these different groups of animals are of the same substance, judging from the description and figures of these authors. Regarding the nature of spindle bodies some authors (MRÁZEK, ORTON, etc., for example) believe that they are degenerated and detached muscle fibres while one of them (YATSU) confirms the blood-cell origin of these enigmatical bodies. YATSU succeeded to discover nearly all stages of transformation from the ordinary red blood corpuscles up to the spindle bodies. It seems, therefore, that there is no doubt about the blood cell origin of spindle bodies so far as *Lingula* is concerned. The reasons from which MRÁZEK, ORTON, etc. induced the view that the spindle bodies originate from the muscles are, perhaps, itemized as follows:

(1) The spindle bodies show a longitudinal striation, similar to those of smooth muscles. (2) Staining reaction of these bodies and muscles resembles closely each other. (3) The length of spindle bodies exceeds  $100\mu$  or more occasionally, and it seems to be difficult to believe that such enormously long cells would be produced from the blood cells. (4) Spindle bodies are found abundantly in those individuals kept for some time in unfavourable condition which may cause the myolysis.

These four items are, indeed, a strong evidence to assert the myogenetic theory, but these are not sufficient to conclude that the spindle bodies must be produced only from muscles. For the purpose of making this point clear I made some observations on the spindle bodies of *Lingula*.

(1) On the striation. YATSU described that the fibres of the spindle bodies are formed from compact bodies which are produced from the cytoplasm of red cells and are a very compact, homogeneous and strongly refractive sphere. I observed also the similar behaviour of blood cells of *Caudina*, as was noted in my previous paper (1937). That these fibres possess properties somewhat different from the myofibrillae may be concluded from the following facts. i. Of the arrangement. Some of the spindle bodies show a specific arrangement of fibres which is not found in the muscles. There are seen occasional cells in which a few fibres are clustered irregularly in their central portion. It is also discernible in some instances that there are two to five, or very occasionally six or more bundles (Fig. 9, o), so arranged as to form various angles with each other.

These irregular arrangements of fibres are not seen in the muscles. ii. Resistance to chemical reagents. In a majority of the cases the fibres of spindle bodies are more or less coarser than the myofibrillae, but they are less resistant to various acid and alkali. Weak alkali such as N/100 NaOH causes the fibres to dissolve. Acetic acid, dilute hydrochloric, sulphuric, or oxalic acid causes also the dissolution of fibres, but in nitric acid (N/10) the spindle bodies are fixed and well preserved. After the dissolution with sulphuric acid there is seen the new appearance of numerous needle-shaped crystals. The spindle bodies are also preserved in the ordinary fixatives such as corrosive sublimate, picric acid, osmic acid, formol, iodine, etc. These bodies are not dissolved in alcohol, ether,

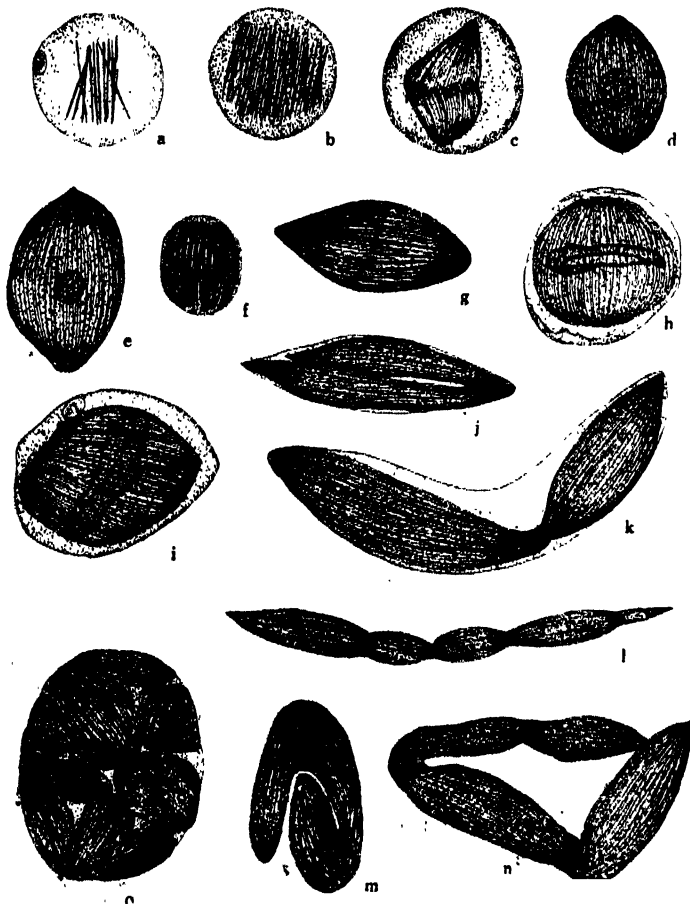


Fig. 9. Various kinds of spindle bodies. ca. 1,200 $\times$ .

chloroform, acetone, xylol, benzol and so forth. These properties are common to those of muscles. iii. As was observed by CORI in *Phoronis* and YATSU in *Lingula*, a series of fine, easily stained refractive granules can be seen arranged on the equatorial surface of spindle bodies, in favourable preparations fixed with formalin and stained with acidulated methyl green (Fig. 9, f, g). The granules are also demonstrable with osmic acid or iodine fixation, though YATSU stated that they are not visible by any other methods than that of acidulated methyl green. In the preparations treated with Cresylechtviolett supravitaly, a disc stained slightly in purplish tinge and penetrated with numerous fibres appears in the equatorial plane of cells (Fig. 9, h, i). This disc cannot be taken as nucleus, since it differs in the chromatic properties from the nucleus. Moreover there is seen frequently a nucleus besides the disc (Fig. 9, i). In the muscle fibres such granules or discs are not discernible.

(2) On the staining reactions. As was pointed out by YATSU, the behaviour of spindle bodies toward the stains suggests that of muscle. In the present observation it is also well in accordance with this statement. Both tissues are positive to eosin, erythrosin, Cresylechtviolett, brilliant cresyl blue, Nile-blue sulphate and so forth. BEST's carmine and Scharlach R. stain the spindle bodies a little. By the methods of GIEMSA, MAY-GRÜNWARD, EHRLICH-BIONDI, etc. they show oxyphilic staining. It is generally accepted that there is found no nucleus in these enigmatical bodies. I succeeded, however, to demonstrate the nucleus of spindle bodies in some favourable preparations stained with Cresylechtviolett, acidulated methyl green and so forth (Fig. 9, a, d, e, i). The nucleus is round or oval, usually single but occasionally two in a cell and measures 4-6  $\mu$  in diameter. The nuclear membrane is distinct and nuclear content is densely granular. The nucleated spindle bodies are figured by MRÁZEK and ORTON (l. c.) but they are interpreted as the leucocytes which have ingested the spindle bodies. The nucleus in that case, therefore, does not belong to spindle bodies, but the leucocytes. There would happen such a case occasionally, but it is difficult to consider from the following basis that all the nucleated spindle bodies are produced in this manner. i. There is no such a large hyaline amoebocyte as is able to gulp the spindle body. As already mentioned above, the hyaline amoebocytes are the smallest cells in the coelomic fluid of *Lingula*, while nucleated spindle bodies are usually several times larger than them. Granulocytes are large enough to ingest small or median sized spindle bodies. It is seen, as a matter of fact, that occasionally the granulocytes contain a single spindle

body (Fig. 8, e). In this case the spindle body is, of course, surrounded with numerous granules which are not to be seen in the spindle bodies. ii. Hyaline amoebocytes frequently possess bean-shaped nucleus, but I failed to find nucleus of such a shape in the spindle bodies. iii. The cytoplasm of nucleated spindle bodies turned, in a majority of the cases, to the fibres thoroughly, and there remains the ordinary hyaline cytoplasm very sparsely in the perinuclear zone. In the case of usual phagocytosis there exists a remarkable extension of ordinary cytoplasm besides the ingested substance.

(3) On the enormous length of spindle bodies. YATSU described the spindle bodies to be vary greatly in size, measuring  $55\mu$  in length on an average, and the longest often attaining twice the usual length. Such enormous length makes it difficult for us to believe that they can be produced from the red blood corpuscles which measure  $10-20\mu$  in diameter. It is not impossible to explain, however, that the spindle bodies of such a large length are formed in the shape of coil in the cell body. There exist, as a matter of fact, frequently the coiled spindle bodies of various complicacy. In some of them the coils begin to disentangle, and turn to a long linear body (Fig. 9, m, n). In such a long spindle body there are found from one to several constrictions from which the bundle of fibres is bent or twisted. When the bundle is folded into two, forming the shape of the letter V or U, there is a constriction at about the middle of it. There exist two constrictions in the case in which the bundle assumes the shape of triangle or letter  $\varphi$ . The bundle which possesses three constrictions does not form tetrangle, but it is folded into four in the shape of the letter W,  $\xi$ , an eddy current etc. Thus the bundle of fibres assumes various complicate forms with the increase of constriction. It seems to me that the bundle of fibres breaks into small pieces from these constrictions, and they take an irregular arrangement at the inside of the cell membrane (Fig. 9, o). These pieces become free when the cell membrane would be burst. If this assumption is true it will be understood that there exists a considerable variation in the size of spindle bodies.

(4) That the spindle bodies are found more abundantly in those individuals which were kept for some time in unfavourable condition is certified by MRÁZEK in *Annelida*, ORTON in *Mollusca* and the present author (1936) in *Echinoderma*. ORTON believes, as mentioned above, that the spindle bodies are produced by muscular degeneration. He made an observation by boring a hole in the shells of oysters to attempt to produce



spindle bodies artificially. Of the 11 oysters bored in this experiment 7 showed spindle bodies, similar to those obtained in gaping oysters. I made also an experiment on the production of spindle bodies of *Lingula* though with failure. The peduncles were torn off from the shell, the fluid of peduncular cavity in which the spindle bodies are found most abundantly was removed by gently pressing the peduncles. These peduncles were kept for several days in fresh water. The muscular wall of them begins to degenerate and soften, and milky fluid of disintegrated muscles flows out from peduncular cavity into the water. A drop of this fluid was examined under the microscope, in expectation of the abundant occurrence of spindle bodies, but there was found very few or nearly none of them. From this result it may be also concluded that the spindle bodies are formed essentially from some other elements than the muscles, at least in the present specimens.

From the facts mentioned above it may be understood that the origin of spindle bodies of *Lingula* can be explained from some other basis than the myogenetic theory, though it is insufficient in data for the present.

#### SUMMARY

1. In the coelomic fluid of a brachiopod, *Coptothyris grayi* we can find the formed elements similar to those of *Terebratalia coreanica* of which I gave some informations in the previous paper.
2. The spindle bodies are found in the body fluid of both animals (*Coptothyris* and *Terebratalia*) upon rare occasions.
3. The body fluid of *Lingula* contains the red cells which were entirely absent in that of *Coptothyris* and *Terebratalia*. The pigment of these cells are, perhaps, a kind of haemoglobin. It crystallized intracellularly in the rhombic tabular shape, and shows the positive reaction to TEICHMANN's test.
4. The red cells contain one or several refringent granules which are positive to supravital staining and Nadi-reaction. The full grown red cells are rather deficient in cytoplasmic inclusions besides these granules.
5. Besides the red cells, hyaline amoebocytes, granular amoebocytes (eosinophils and basophils) and spindle bodies are found in the body fluid of *Lingula*.
6. Some description and discussion were made on the structure, properties and genesis of the spindle bodies.

In conclusion the author wishes to express his hearty thanks to Dr. S. HATAI for his interest during the course of the work and kindly reading of the manuscript sparing his precious time. Acknowledgement is also due to Mr. K. HATAI for his kind identification of specimens, and to Mr. K. BABA who kindly sent material for the author's use.

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# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## IX. ON THE COELOMIC CORPUSCLES OF AN EARTH WORM, *PHERETIMA SIEBOLDI*, HORST

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(With four figures)

(Received August 23, 1937)

Recently HATAI (1931) published information regarding the rediscovery of *Pheretima sieboldi* which had been described first by HORST about half a century ago (1883). I was fortunately bestowed with about twenty living specimens of these semigigantic earth-worms by Mr. J. ISHIKAWA who collected them in the vicinity of Uwajima City, Ehime Prefecture, Japan. When one handles roughly or places them in the vapour of alcohol or acetic acid they spurt a large amount of coelomic fluid from the dorsal pores so intensely that it reaches to the height of about twenty inches or more. Similar fluid is also copiously discharged from the mouth, and it is, on examination, found to be coelomic fluid with all the usual cells. These facts led me to commence some observations on the coelomic fluid of the present specimens.

### THE CELLULAR ELEMENTS OF THE FLUID

I recognized four distinct kinds of cells in the perivisceral fluid; 1) lymphocytes, 2) granulocytes, 3) lamprocytes, and 4) linocytes. In addition to these elements, the perivisceral fluid contains occasionally chloragocytes, peritoneal cells, and spindle bodies. As to the cells of the first two kinds the appearance and structure are essentially similar to those of *Drawida hattamimizu* HATAI, of which some accounts had been given in my previous paper (1934) so I will give only the description concerning the reaction of these cells to the various stains and reagents.

1. Lymphocytes. The cells of the monocyte-type are also grouped under this name. The lymphocytes are positive to the vital- and supravital staining with various ordinary dyes. These cells contain a few fine granules stainable with Sudan III or Scharlach R. Indophenol blue

synthesis is positive. The granules or rods which show an affinity to Janus green B are remarkably abundant in the perinuclear region.

2. Granulocytes. These cells are the next abundant in the perivisceral fluid. Most of them possess the eosinophilic granules, but there are seen those with basophilic granules occasionally. The granules of the latter type are somewhat coarser than those of the former. The properties of eosinophilic granules to the various dyes and reagents are as follows.

The granules are positive to the supravital staining. These dyes are, to name them in the order of the intensity of staining, brilliant cresyl blue, Nile-blue sulphate, Janus green B, neutral red, methylen blue, erythrosin, etc. The granules are not stained with Sudan III, but slightly stained with Scharlach R. A long exposure of the cells to the osmic acid causes a little blackening of granules. The Nadi-reaction is intensely positive. The granules are resistant to alcohol, ether, chloroform, benzol, acetone, and xylol, but are changed more or less in their appearance by these reagents; namely, they become less refractive, and somewhat irregular in shape. They are fixed with the ordinary fixatives such as absolute alcohol, acetone, corrosive sublimate, uranium nitrate, formalin, iodine, acetic acid, picric acid, nitric acid, etc., but soluble in oxalic acid,

caustic soda etc. MILLON's reaction is slightly positive. From these reactions it is evident that they are not composed only of lipid but also of protein. It is generally accepted that the granules of human eosinophils are formed with a protein nucleus enveloped in a lipid substance. Such structure may be, I suppose, the case in the present specimens too.

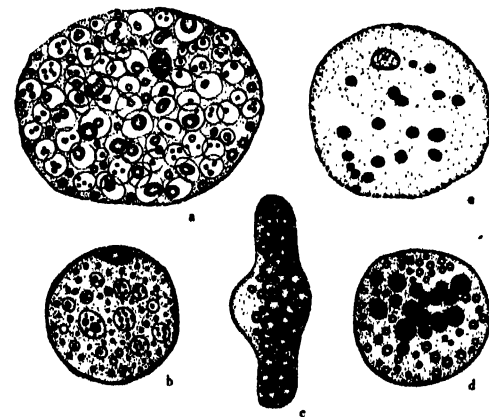


Fig. 1. Lamprocytes. ca. 900 $\times$ .

a, b. Lamprocytes with globules which contain small and refringent bodies.

c. Profile of a lamprocyte.

d. Lamprocyte stained supravitally with brilliant cresyl blue.

e. Lamprocyte fixed with a weak solution of corrosive sublimate.

3. Lamprocytes. These cells are the most abundant of all the elements in the fluid, and are relatively large, oval or circular discs with a remarkable bicon-

vexity, measuring  $26\mu$  on the average (Fig. 1). They are bound with a distinct pellicle and incapable of forming pseudopodia. The cytoplasm is clear and transparent; it is embedded with colourless or slightly yellowish and circular globules, many of which contain small but refringent bodies (Fig. 1, a, b). The number of these bodies in a globule is limited to only one in the reports of many authors, so far as I am aware, but there are found occasionally six granules or more in the present specimens.

In a majority of lamprocytes there exist two kinds of globules which are distinguishable from one another in their size and properties to dyes and reagents. A kind of globules is several times larger than another, usually contains the refringent bodies, and is positive to supravital staining with brilliant cresyl blue, Nile-blue sulphate, neutral red, Cresylechtviolett, methylen blue, etc. Another kind of globules is small, contains no refringent body, and difficult to stain supravitaly with the dyes just mentioned above. The former is from several to twenty or more in number but the latter is innumerably abundant. The refringent bodies have most intensive affinity to the dyes, and, accordingly, they are stained deeply even in the case that the colour of dye begins to appear very slightly in the fluid in which those bodies are suspended. When one may apply one per cent solution of corrosive sublimate to the lamprocytes, all the small globules disappear immediately while the large vacuoles remain in situ, though they show more or less shrinkage and, consequently, an irregular contour. The small globules dissolve in alcohol, when they are fixable with corrosive sublimate, as was mentioned just above. In the smear preparation fixed with either ethyl- or methyl alcohol and stained by GIEMSA's method the most globules of lamprocytes are solved away, and become visible as a single, occasionally double nuclei, which are usually concealed with globules, and cytoplasm with reticulation in which the globules were sustained. Such structure resembles very much to the large granulocytes of *Physcosoma* of which I described and figured in my previous paper (1937), and it suggests a close relationship between these two animals. There exist a few meshes which are filled with basophilic substance. These are, perhaps, incompletely dissolved large globules. When brilliant cresyl blue in absolute alcohol is added to the edge of the cover glass, the small globules burst and disappear immediately. The large globules are stained deeply with the dye, but they disappear also after a few minutes.

The globules of both types behave similarly to the various reagents except the case for the corrosive sublimate, as was just mentioned above.

They are fixed with osmic acid, picric acid, phosphomolybdic acid, uranium nitrate, etc., but they are disintegrated in potassium iodide, formol, boiled water, etc. Scharlach R. stains them slightly, but they are negative to Sudan III and Nadi-reaction. MILLON's, biuret, or ninhydrin reaction is also negative. Various solvents of fats such as ether, chloroform, acetone, benzol, xylol etc. cause the globules to disappear. Nitric acid (strong) causes the cells to swell, the globules also swell up and burst. Hydrochloric acid, sulphuric acid, acetic acid, oxalic acid, etc. have the same effect. In potash or caustic soda the entire cell swells, and the contents disappear instantaneously on the arrival of the reagent. BEDDARD (1895) had suggested that the granular corpuscles (i. e. lamprocytes) serve as excretory cells. It seems to me that the fragility of the globules endorses his view.

4. Linocytes. There are found numerous linocytes of various shapes in the perivisceral fluid of the present specimens while those of *Drawida*

were very sparse in number and atypical in shape (Fig. 2). The great majority of the linocytes of *Pheretima sieboldi* are spherical, of about the same size of lamprocytes, but some of them are as small as the lymphocytes. Between these two extremes there are all degrees of size. The young linocytes are distinguishable from the lymphocytes by the presence of a single, occasionally two or three, large vacuoles which have pushed the nucleus to one side (Fig. 2, a-k). In the middle of vacuole there is seen frequently a

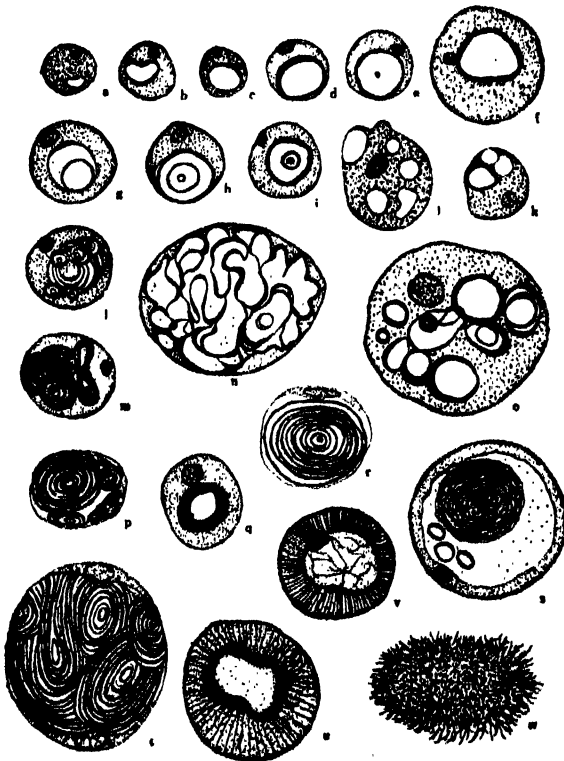


Fig. 2. Various kinds of linocytes, ca. 900x.

refrangent concrement (Fig. 2, e). The nucleus is usually oval, and lies in the cytoplasm which is thicker in its neighbourhood than elsewhere. The cytoplasm is finely but regularly granulated. The vacuoles gradually increase in size by the union of smaller vacuoles with one another. Then the outline of the united vacuoles becomes refrangent and forms a circular ring, and from this ring fine, coiled threads are produced. These facts accord well with the description of BENHAM (1901) who has made informations on the blood of some acanthodrilids. The young linocytes possess usually a single ring or thread-coil, but there exist several independent thread-coils in the full grown linocytes (Fig. 2, o). These coils become to be entangled with one another and show a very complicated appearance which is similar to the myelin form (Fig. 2, n). There are seen infrequently those linocytes in which the thread is indistinct, but a series of radiating lines occupy the peripheral region of the cells (Fig. 2, u, v).

Action of reagents. i. Gentian violet stains the threads and the fluid in the vacuoles very rapidly a bright blue, as was noted by BENHAM.

ii. Nile-blue sulphate stains the threads a deep purplish blue but the fluid is hardly stained with the dye.

iii. Cresylechtviolett stains the threads a blue violet and the fluid of vacuoles slightly pink.

iv. Iodine stains the threads a bright yellow, much more deeply than the cytoplasm.

v. The threads are very slightly and diffusely stained with neutral red, brilliant cresyl blue, methylen blue, Cresylechtviolett, etc.

vi. With Scharlach R., the threads are stained an orange yellow.

vii. BEST's carmine stains some granules in the cytoplasm of linocytes.

viii. In acetic acid the thread gradually swells and loses its distinctness till it finally disappears.

ix. Oxalic acid causes the thread to swell and deform till it is reduced to granules.

x. In phosphomolybdic acid the thread becomes curly, but further change is not visible. The action of picric acid is similar.

xi. Nitric acid. The cell shows opaque granulation that conceals the thread within. Strong nitric acid causes the thread to reduce to the granules though undissolved. The action of hydrochloric and sulphuric acid is similar.

xii. In caustic soda (N/10) the cell swells, the thread loses its refringency and distintegrates gradually into fine granules.

xiii. Absolute alcohol (cold) does not dissolve the thread, though it



shrinks the thread a good deal. Hot alcohol, however, dissolves the thread easily.

xiv. In ether the thread shows no remarkable change. The action of chloroform and benzol is similar.

xv. Aceton causes the thread to swell and turn its contour irregular. In aceton occasional cells take an appearance like the bur of chestnut, with numerous, fine hair-like protuberances on the cell-surface (Fig. 2, w). Xylol has the same effect.

xvi. MILLON's, biuret, or ninyhydrin reaction is negative.

From these reactions it is supposed that the linocytes contain some lipid substance in relative abundance.

5. Other cells free in the perivisceral fluid. In addition to the aforementioned elements, the perivisceral fluid contains detached chloragocytes, peritoneal cells, and spindle bodies. Regarding the first two cells I have no additional datum to the description found in my previous paper. The presence of spindle bodies in the body fluid of *Annelida* was described by MRÁZEK (1910). According to his description these bodies are hyaline and refractile, variable in length and form, usually of spindle-shape, always flat and plate-like, with a fibrillar structure. These are believed by MRÁZEK to be portions of the longitudinal muscle layer thrown off into the body cavity. I found spindle bodies in only one of about twenty worms (Fig.



Fig. 3. Spindle bodies found in the coelomic fluid of an earth worm. ca. 1,200 $\times$ .

3), and they were essentially similar in shape and properties to those of *Lingula*, I failed to detect any genetic relation between the spindle bodies and linocytes, though these two cells are alike in the point that they possess more or less fibrillar structure.

In the coelomic fluid which is yellow in colour there are certain cells known as eleocytes. That these cells are found in the worms which contain milky white body fluid is also reported by BENHAM.

The characteristic feature of these cells, according to his report, is the presence of numerous, clear, colourless globules of oil, which crowd in the cytoplasm, and — unlike those recorded

for *Allolobophora* -- are not limited to the periphery, but occur throughout the entire depth of the cell. KINDRED (1929) gave no account on these cells of *Pheretima indica*, and the present author failed also to detect them in *Drawida*. In the present investigation it is also difficult to distinguish the eleocytes from the lamprocytes. According to BENHAM the difference of the two types of cells is as follows: The eleocytes possess the cytoplasm filled with highly refringent oil globules while that of the lamprocytes is occupied by numerous vacuoles, each of which usually contains a small, high refringent granules. I can not think that such difference is sufficient to distinguish the two kinds of cells. For there is every transitional stage between these two types. This fact was recognized also by BENHAM himself. He noted that there is little doubt as to the relation of one to the other, though he was unable to say which of the two cells is derived from the other, or which is the earlier stage in the history. BENHAM recorded, on the other hand, the difference of behaviour of the two cells to chemical reagents. According to him, the globules of eleocytes are soluble in alcohol and ether, but insoluble in nitric acid and hydrochloric acid, while the granules of lamprocytes show just the reverse relation. In the present investigation all globules and granules were soluble in these reagents, as was already mentioned above.

The body fluid is opaque white when fresh, resembling milk in appearance; it very soon becomes coagulate, and in a few minutes hardens to form a dirty yellow mass. As was described by BENHAM in the case of acantholrilids, that hardened mass is so tenacious that it clogs scissors, sticks fingers together, and pastes a cover glass on the slide unremovably. By the microscopic observation of such mass there are found coelomic corpuscles which are deformed into a spindle shape by the compression of coagulated fluid (Fig. 4). The coagulated mass is stained intensely by

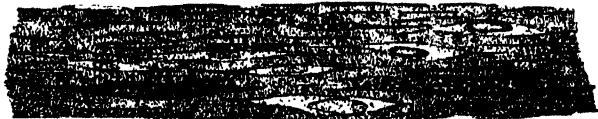


Fig. 4. Coagulated body fluid. ca. 600 $\times$ .

WEIGERT's method of fibrin staining. It is possible to withdraw the body fluid with hypodermic syringe without exposing it to the air. The cover glass preparations are made with this fluid. In such preparation the fluid

will be kept for a while without coagulation, and the various coelomic corpuscles which are in suspense or movement will be seen in it. When alcohol is applied to the edge of the cover-glass, the fluid begins to coagulate, as soon as the arrival of alcohol, and turns to a dirty, opaque and hard mass. The coelomic corpuscles change themselves into a spindle or a ramified shape by the compression of the coagulated fluid. The coagulation of the fluid takes place also after the elimination of formed elements. It is evident, accordingly, that the coagulation of the fluid has no or little relation to the coelomic corpuscles, although some of them (chiefly lymphocytes and granulocytes) show the agglutination on the outside of animal body. Why some kinds of earth worms possess the coagulable body fluid while others have non-coagulable one? This question remains to be solved.

#### SUMMARY

1. The body fluid of *Pheretima sieboldi* contains four types of leucocytes; the lymphocytes, the granulocytes, the lamprocytes, and the linocytes. In addition to these elements there are found occasionally chloragocytes, peritoneal cells and spindle bodies. The latter are essentially similar to those of *Lingula*.

2. Lamprocytes contain large and small globules in various numbers. The former are fixed with corrosive sublimate and stained easily with various vital dyes while the latter disappear by the fixation with that chemical and are difficult to be stained with vital dyes.

3. Linocytes, from the very young to the full grown, are seen in the peritoneal fluid. The threads of linocytes show some lipid reactions.

4. The coagulation of the body fluid is essentially due to that of plasm; namely the body fluid which contains no cellular elements is coagulable strongly.

The author takes this opportunity of acknowledging the kindness of Dr. S. HATAI, who read and criticized the manuscript of this paper. An acknowledgement is also made to Mr. J. ISHIKAWA for his kind bestowal of the valuable material.

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# HISTOLOGICAL OBSERVATIONS ON THE BARBELS OF FISHES

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(With eleven figures)

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## INTRODUCTION

One of the most remarkable external appendages existing on the head of the fish is the barbel. There are found about 60 species in Japan of such fishes possessing this organ. Most of them are occupied by the cyprinoid fishes, catfishes and grenadiers.

The fishes possessing barbels are generally bottom feeders of rather sluggish habit and in some cases they are nocturnal feeders. The function of this organ serving as an external gustatory was shown by HERRICK ('02) in American catfishes. In my previous papers (SATÔ, '37a, b, c), the gustatory function of the barbels of Japanese goatfish and sea catfish was described, and it was then found that the barbels seem to be useful and important in the daily activity of such fishes.

While the histological observations of the barbels are known through the works of WRIGHT ('84), HERRICK ('01), OLMSTED ('20), MAY ('25) and SATÔ ('37a, c), no one, so far as I know, has attempted to make comparative observations on the structure of this organ. The purpose of the present research is to determine whether or not similar histological structure of the barbels exist in various kinds of fishes.

The material used in this investigation was taken from the following localities: Simoda, Sagami, and Suruga Bays; Lake Kasumigaura in Ibaragi-ken; the Inozawa River near Simoda; the Ôsio River in Hukusima-ken; the Takase River in Kumamoto-ken. For fixation, Bouin's and Zenker's fluid, formol-Zenker and strong Flemming's solution were tried. The sections were cut  $10\mu$  thick, and were differentiated by the stains of widest application.

Here I offer my sincere gratitude for the kind instruction and encouragement given to me in this work by Prof. Dr. S. HATAI. And it is my pleasure to express my hearty thanks to Mr. TAKANAGA MITSUI for having given me facilities for excuting this investigation.

## RESULTS OF THE OBSERVATIONS

1. *Pristiophoridae*.

*Pristiophorus japonicus* GÜNTHER, the saw-shark, is found rather sparsely along the shores of Japan. The material for my investigation was obtained from Sagami Bay. This fish has the snout produced into a long flat blade, bordered on each side by a row of rather small sharp enameled teeth and is provided on each side with a grayish, unbranched barbel. This barbel is somewhat depressed dorso-ventrally and is about 50 mm. long in the adult.

This barbel is remarkable in the following characters: i. the dermal spines cover the surface of the epidermis; ii. the cutaneous taste buds are not distributed in the epidermis (Fig. 1). This dermal spine has its base in the dermal layer and raises to the outer surface of the epidermis. The epidermis consists of oval shaped epidermal cells, but the taste buds and mucous cells are not buried in it. At the central core of the dermis which is composed of connective tissue, is a rod of cartilage which stretches from the base of the barbel to its distal end. The nerve trunks and large blood vessels are found on both sides of this rod.

2. *Siluridae*.

This group has the skin naked or imperfectly mailed and the barbels on the head are well developed. The fishes belonging to this group are generally bottom feeders and seem to search for their food chiefly by dragging their barbels on the bottom. The following four species investigated by me are similar in structure: *Parasilurus asotus* L.<sup>1)</sup>, *Pelteobagrus nudiceps* (SAUVAGE), *Pseudobagrus aurantiacus* (T. & S.), *Liobagrus reini* HILGENDORF<sup>2)</sup>. The structure of the barbel of *Liobagrus reini* is here described as the representation of this group.

This fish possesses eight barbels on the head: the nasal and maxillary barbels each have one pair and the mental ones have two pairs. All barbels are similar in form and structure. The epidermis is composed of stratified epidermal cells among which the cutaneous taste buds, mucous and club-cells (Kolbenzelle) are found (Fig. 2).

The taste buds are flask-shaped, about  $80\mu$  in height and about  $40\mu$  in maximum diameter. The buds are generally more populated on the distal parts of the barbel than the proximal ones. Since the structure

<sup>1)</sup> According to ATODA ('35), this fish possesses two pairs of barbels in the adult stage, but in younger bears three pairs.

<sup>2)</sup> It is said that this fish lays eggs on the bottom of the clear stream, and watches and protects them (UCHIDA, '33. Zool. Magazin., 45).

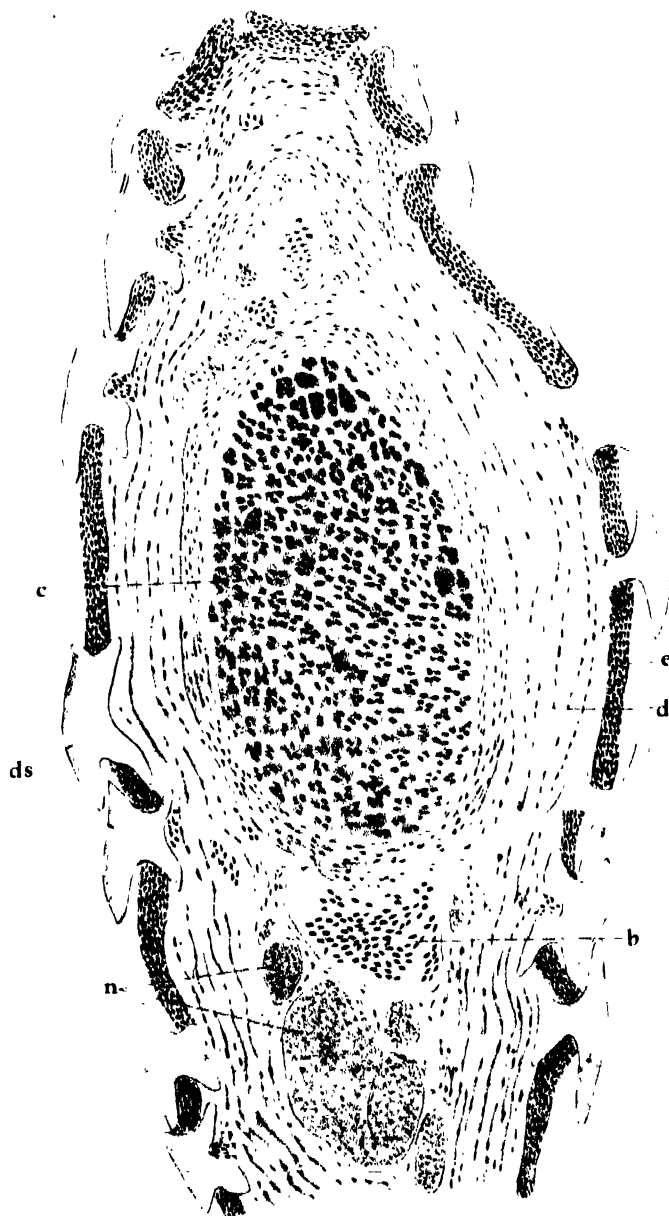


Fig. 1. Transection of a barbel of *Pristiophorus japonicus* GÜNTHER.  $\times 50$ .



of the taste bud is essentially like that of American catfishes<sup>1)</sup>, I may dispense with a description of it.

The mucous cells develop fully in the upper half of the epidermis, and their distal end occupies the greater part of its surface, scarcely leaving space for the epidermal cells. They are elongated and contain the mucous substances which are stained deeply with Delafield's haematoxylin.

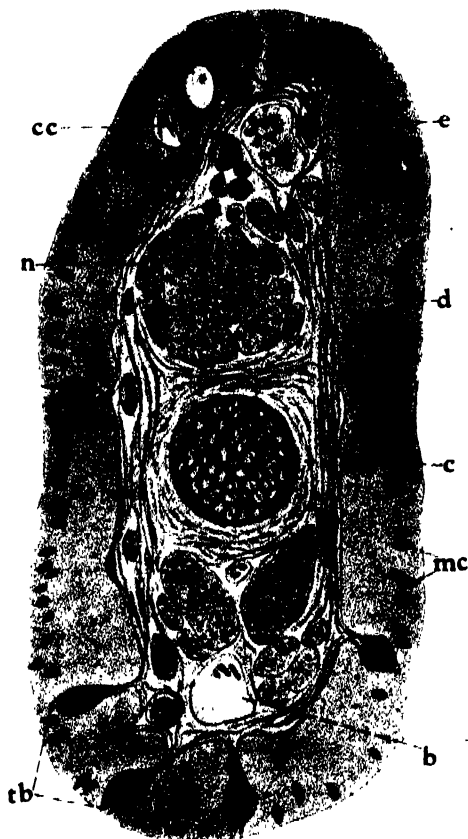


Fig. 2. Cross section of a barbel of *Lior bagrus reini* HILGENDORF.  $\times 100$ .

The club-cells are elongated oval in shape and are found sparsely among the epidermal cells. The inner structure which is surrounded by epidermis is quite similar with the Japanese sea catfish (SATÔ, '37c). A rod of cartilage occupies the center of the dermis, and large nerve trunks rest at the dorsal and ventral sides of this rod and stretch from the base of the barbel to its end, parallel with the rod. In its course, numerous side branches are given off through the connective tissue, reaching to the base of the cutaneous taste bud. The blood vessels are also found in the dermis.

### 3. Cobitidae.

The loaches are small fishes, all less than a foot in

length, mostly living in small streams and ponds, where they move like eels.

I have not yet an opportunity to observe the other loaches avoiding *Misgurnus anguillicaudatus*, however, the other barbels also seem to show similar structure with that of the loach described here.

*Misgurnus anguillicaudatus* (CANTOR) is a widely distributed species in Japan and ten barbels are found about the mouth: six are on the

<sup>1)</sup> Full descriptions were given by HERRICK ('01), OLMSTED ('20) and MAY ('25).

upper jaw and four on the lower.

The epidermis of the barbel is composed of oval-shaped epidermal cells, mucous cells and taste buds. The structure was well described by MIYADI ('29), I shall confine my remarks to the dermal part of the barbel.

A cartilaginous rod rests at the center of the dermis, and numerous melanophores surround this rod (Fig. 3). The smooth muscle layers cover

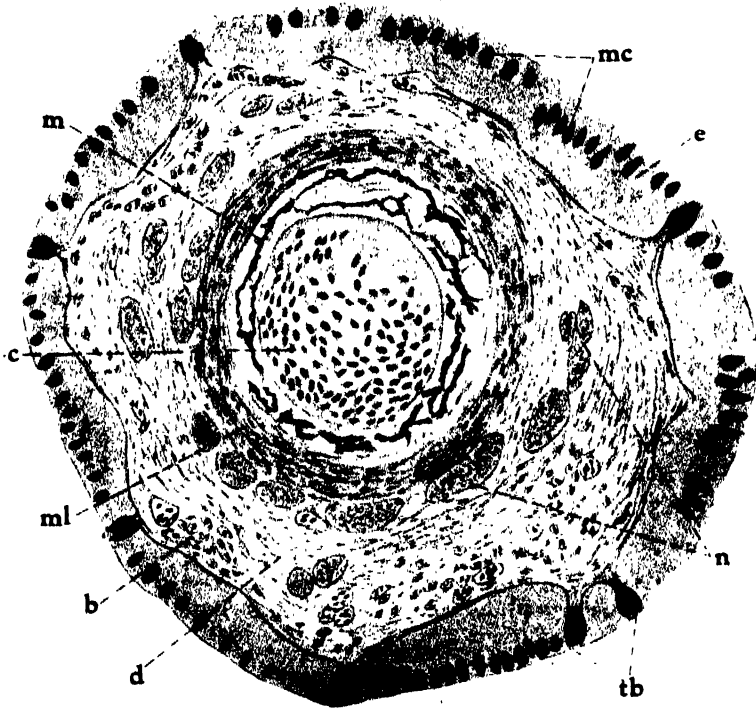


Fig. 3. Cross section of a barbel of *Misgurnus anguillicaudatus* (CANTOR).  $\times 100$ .

these pigment cells, and the bundles of nerve fibers scatter in the connective tissue in contact with these muscle layers. In this barbel, however, the nerve bundles do not come together to a large mass as that of the catfishes and goatfish. The blood vessels and melanophores are also found in the connective tissue lying closely to the epidermis.

#### 4. Cyprinidae.

The small barbels are found on the upper jaw of some species of this family. Among them, I observed the barbels of the following three species: *Cyprinus carpio* L., *Hemibarbus barbus* (T. & S.) and *Pseudogobio esocinus* (T. & S.), and I found that all these barbels show a similar

pattern in the structure with that of the carp described here.

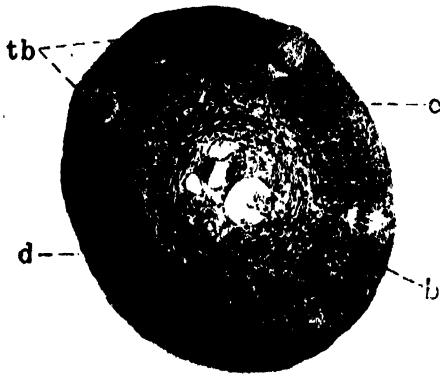


Fig. 4. Cross section of a barbel of the carp.  $\times 90$ .

*Cyprinus carpio*, the carp, is the most common fish among this family, preferring shaded, tranquil and weedy waters with muddy bottom. This fish possesses two pairs of barbels on the upper jaw, in this respect the present species is a remarkable exception among the cyprinoid fishes.

The most remarkable aspect in the structure of this barbel is the lack of a cartilaginous rod resting at the center of the dermis (Fig. 4). This part is

occupied by the blood vessels and bundles of nerve fibers. These nerve bundles divide in many small branches and reach to the bases of the cutaneous taste buds which are buried in the epidermis. However, the club- and mucous-cells are not found in the epidermal layer, so far as my investigation goes.

##### 5. Polymixidae.

*Polymixia japonica* STEINDACHNER is obtained from the rather deep water of southern Japan. This fish is plainly colored without red, having rather smooth scales, and at the chin are two long barbels which look remarkable like those of the family of Mullidae. However, the specialized feelers at the chin are different in their supporting bone and their structure. In *Polymixia*, each barbel is suspended from the hypohyal; while in the goatfish, each barbel is suspended from the tip of a slender projection of the ceratohyal. In the structure of the barbel, the following differences are also observed.

The cutaneous taste buds buried in the epidermal cells are not so large and abundant as that of the goatfish (Fig. 5). This organ is flask shaped like that of the carp and catfish, and less spherical than those found in the goatfish. Furthermore, the rod resting at the center of the dermis does not consist of cartilaginous tissue, but it is composed of the striated muscle tissue mingled with connective tissue. Among the dermis surrounding this rod, there are large nerve trunk and blood vessels, like that of the other barbels.

Though this barbel is different from that of the goatfish in its structure, it seems to be necessary to the present fish in cognizing hidden food like the barbel of the goatfish (SATÔ, '37a, b).

#### 6. Haemulidae.

*Hapalogenys nigripennis* (T. & S.) is one species having the barbels in this family. Fishermen say that this fish lives in rocky sea bottom. The

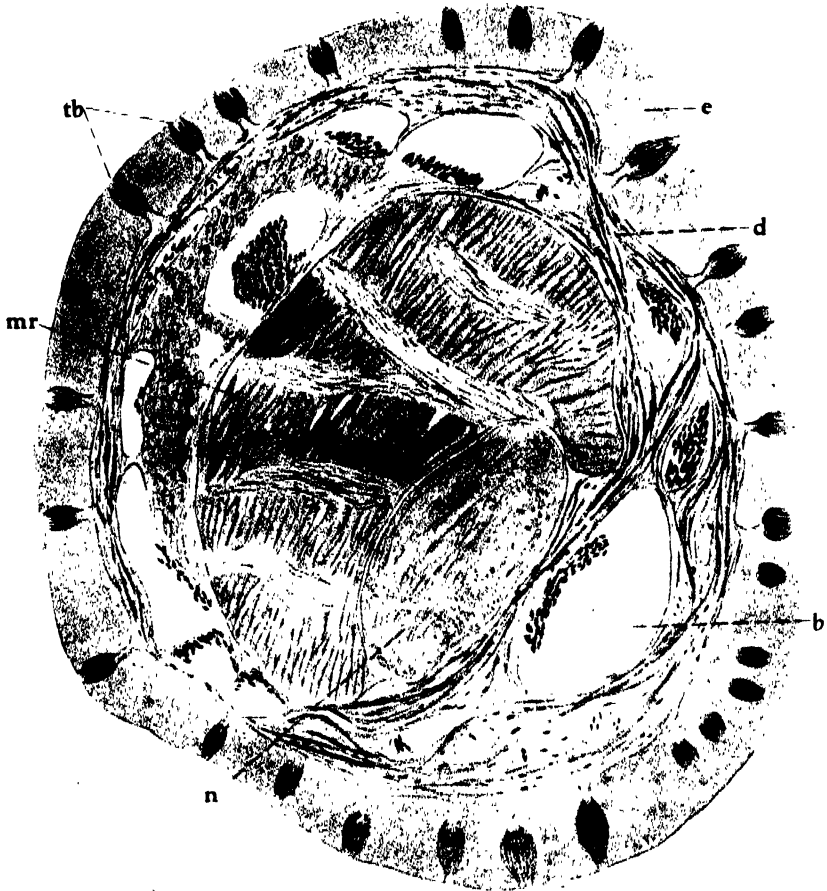


Fig. 5. Cross section of a barbel of *Polymixia japonica* STEINDACHNER.  $\times 50$ .

present fish possesses a large number of rather short, dark-colored barbels at the chin (Fig. 6).

This barbel shows the structure similar with that of the carp. It does not have a cartilaginous rod in the dermis, and this part is occupied by the connective tissue which is rich in the interstitial substances (Fig. 7).

The blood vessels and pigment cells are also buried in the connective tissue closely underlying the epidermis, among which the spindle shaped taste buds are found, but the mucous- and club-cells are not distributed in it.



Fig. 6. Side view of the head of *Haplo- genys nigripennis* (T. & S.), showing the barbels at the chin. ca.  $\times 1/3$ .

#### 7. Scorpaenidae.

*Apistus evolans* JORDAN & STARK is a disagreeable fish for the stings from its dorsal spines, which are provided with poison gland. This fish is a bottom feeder and remains most of the time quietly on the muddy bottom.

Three barbels are found at the chin: one rests at the tip of the lower lip and other two exist on the each side of it. These barbels are about 10 mm. in the length and are without any colors. The structure of the barbel is akin to



Fig. 7. Cross section of a barbel of *Haplo- genys nigripennis*.  $\times 100$ .

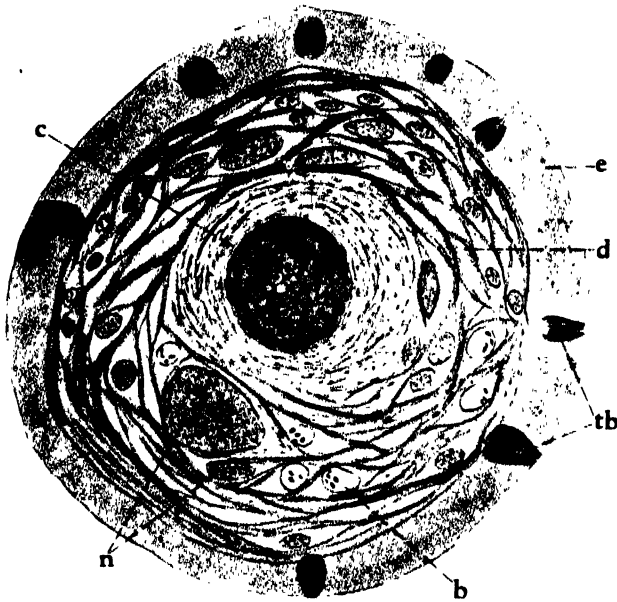


Fig. 8. Cross section of the barbel of *Apistus evolans* J. & S.  $\times 200$ .

that of the loach, except that the mucous cells are not so numerous and the pigment cells are not found in the dermis (Fig. 8).

#### 8. Peristediidae.

This is a group found in the deep water of southern Japan. The following descriptions are based on *Peristedion amiscus* JORDAN & STARK obtained from Suruga Bay. This fish is a sea-robin, much depressed, with flat head, and a large number of barbels at the chin. Among these barbels, a pair at the corner of the lower lip is the largest and is provided with small barbels (Fig. 9).

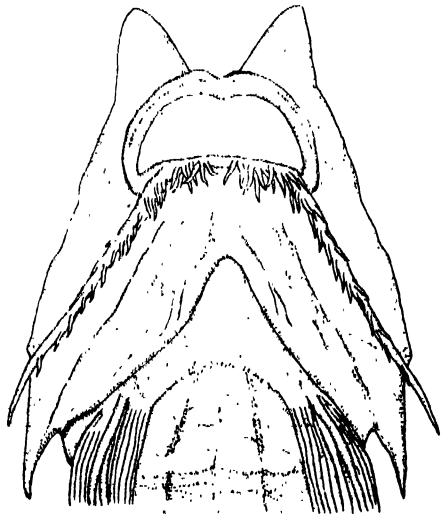


Fig. 9. Ventral view of the head of *Peristedion amiscus* J. & S., showing the barbels at the lower lip.

The structure of the barbel (Fig. 10) is similar with that of the foregoing species. However, the cutaneous taste buds are rather small, about  $30\mu$  in height and about  $20\mu$  in width, and increasing their numbers towards the side small barblets.

*P. orientale* T. & S. and *P. engyceras* GÜNTHER possesses the barbels which are essentially like that of the sea-robin described above.

#### 9. Brotulidae.

*Brotula multibarbata* T. & S. inhabits in the sea of southern Japan and seems to belong to the bottom feeders. This fish resembles the sea catfish in external form, but it does not aggregate in one group and is not provided with any poisoned spines. Twelve barbels are found about the

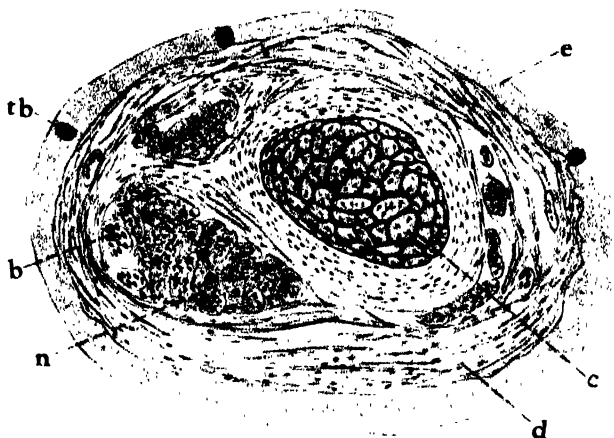


Fig. 10. Cross section of a barbel of *Peristedion amiscus*.  $\times 100$ .

mouth: the nasal barbels have one pair; maxillary with two pairs, and the mental have three pairs. These barbels are about 15 mm. in length in a specimen about 350 mm. long and are tinged with brown. This barbel is akin to that of *Apistus evolans* described above in its structure, but the cutaneous taste buds are found more abundantly in the epidermis.

#### 10. Gadidae.

I have examined the barbel of *Physiculus japonicus* HILGENDORF which is a codfish with a small barbel at the tip of the lower lip. The structure of this barbel exhibits no remarkable deviation from that of the foregoing species, *Brotula*.

#### 11. Macrouridae.

This group generally comprises the deep-sea fishes, and is usually large in size, with rough and spinous scale, dull gray or black in color. Head

is formed much as in the codfishes, but the snout frequently projects more or less beyond the mouth. A barbel is usually found at the symphysis of the lower jaw.

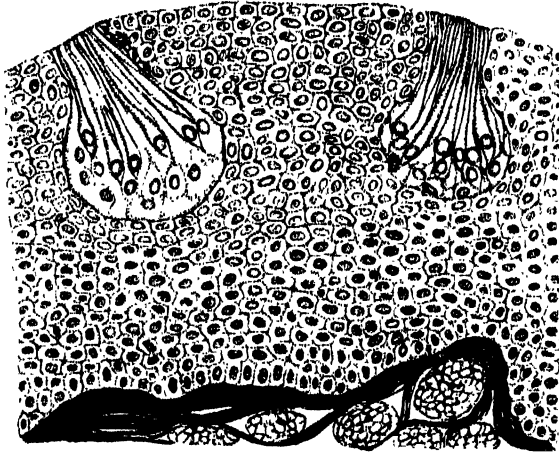


Fig. 11. Transection through the cutaneous taste buds which are buried among the epidermis of a barbel of *Coelorhynchus japonicus* T. & S.  $\times 240$ .

I observed the barbel of *Coelorhynchus japonicus* T. & S. which was obtained from Sagami Bay. This barbel is akin to that of the above mentioned codfish in its structure. However, the cutaneous taste buds are not set directly on the dermal papillae, but are separated from them by several layers of cells of the lower layers of the skin (Fig. 11).

#### SUMMARY AND CONCLUSIONS

I observed the structure of the barbels of 18 species belonging to 11 families in this paper. From these results described in my present and previous reports (SATÔ, '37a, c), the barbels investigated may be summarized as follows:

- I Group. -- the epidermis lacks the cutaneous taste buds. *Pertstrophorus japonicus*.
- II Group. taste buds buried in the epidermis.
  - A. a rod of cartilage does not rest at the dermis -- carp type.  
*Cyprinus carpio*, *Hemibarbus barbus*, *Pseudogobio esocinus*, *Haplogynys nigripennis*.
  - B. a rod of cartilage rests at the dermis.
    - i. catfish type -- *Parasilurus asotus*, *Pelteobagrus nudiceps*, *Pseudobagrus auranticus*, *Leobagrus reini*, *Plotosus anguillaris*.



- ii. goatfish type - *Upeneoides bensasi*.
  - iii. loach type - *Misgurnus anguillicaudatus*, *Apistus evolans*, *Peristedion amiscus*, *P. orientale*, *P. engyceras*, *Bortula multiharbata*, *Physiculus japonicus*, *Coelorhynchus japonicus*.
- C. a rod resting at the dermis consists of striated muscle, Polymixia type — *Polymixia japonica*.

As summarized above, the saw-shark is a remarkable exception in the respect that the taste buds are not found in the epidermis of the barbel. Accordingly, it is uncertain whether this barbel serves as an organ to recognize the food. But, I am inclined to think that the barbels belonging to II Group may function as an external gustatory organ. Because, these barbels are different in the structure of dermal parts, but are almost similar in the structure of the epidermal part, among which the cutaneous taste buds are buried. And it may be concluded that the barbels of the fishes belonging to the same family show a similar pattern in their structure.

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#### ABBREVIATION OF LETTERS

- |                        |  |
|------------------------|--|
| b. blood vessel.       | m. melanophores.                         |
| c. a rod of cartilage. | mc. mucous cells.                        |
| cc. club-cell.         | ml. muscle layer.                        |
| d. dermis.             | mr. a rod consisted of striated muscles. |
| ds. dermal spines.     | n. bundles of nerve fibres.              |
| e. epidermis.          | tb. cutaneous taste buds.                |

# STUDIES ON THE PHYSIOLOGY OF CILIARY MOVEMENT

## III. EFFECT OF ADRENALINE<sup>1)</sup>

By

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(With six figures)

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The present paper constitutes a part of a series of papers dealing with the effect of chemicals and drugs upon the ciliary movement. The main purpose of studying the effect of these substances is to find, if possible, a clue for the physiological analysis of the mechanism of the ciliary movement. Substances having known chemical and physiological effects upon the vital processes will be used.

The movement of cilia has often been compared with that of the cardiac muscle, but the mechanism of the former is not being intensely studied at present, while the latter is the subject of innumerable investigations. Some of the drugs that will be used in the present experiment are important and are attracting the attention of biologists from various points of view, especially, for instance, from the viewpoint of the theory of neurohumor, which is being established on broader and broader grounds of facts which are rapidly increasing.

The distribution and the physiological significance of these substances have been studied mainly in Vertebrates, but some of them are found and have been studied also in certain Invertebrates, as will be mentioned later. These substances are believed to be secreted by nerve endings and play an important rôle in transmitting the nerve impulses from the nerve to the effector. The problem is, therefore, important and interesting not only from the viewpoint of the physiology of ciliary movement, but also from the viewpoint of comparative and general physiology. A review of the literature on the subject and a discussion of the problem will be given at greater length under the heading of comparison and discussion.

### MATERIAL AND METHOD

The material used in this and subsequent experiments is the excised

<sup>1)</sup> Contribution from the Oceano-chemical Institute of the Tôhoku Imperial University, Onagawa, Miyagi Ken.

gill tissue of the oyster, *Ostrea gigas* THUNBERG. One of the shell valves was removed from the animal with an oyster knife, care being taken not to injure the gill, and the gill laminae were cut with a pair of fine scissors, close along the line of attachment of the latter to the body, and were placed in a Petri dish containing sea-water. The gill at first may be covered with foreign matter entangled in the mucous secretion of the animal. On being allowed to stand for some time in the clean sea-water, however, the tissue gradually gets rid of the adhering matter, and may begin to crawl, so to speak, in the container. This is due to the counter action of the ciliary current caused by the movement of cilia over the surface of the gill tissue.

Differentiation and arrangement of cilia in the gill vary according to the groups or species of lamellibranchs, and have been studied by many investigators. The works of PELSENEER, RICE and RIDWOOD among others may be referred to in this connection, and I too have pointed out in an earlier paper the phylogenetic importance of the structure of the gill in *Pecten yessoensis* JAY. In the present paper, it is sufficient to note that the surface of the gill lamina is covered with ciliated epithelium and that the current is caused by the movement of the cilia over its surface. When the gill is excised and cut into pieces of proper size and form, the pieces proceed almost in a straight line. In our experiments, the gill piece was made oblong, a few millimetres on each side. The gill lamina is plicated, and cilia are arranged along the plicae, and the effective stroke of the ciliary motion takes place parallel with the plicae from the dorsal side to the ventral free margin of the lamina. Mucus and foreign substances may adhere to the gill tissue at first, as has been mentioned above, and the ciliary movement is more easily observed when this adhering matter is removed, and carried away by the current. The gill pieces, prepared as mentioned above and placed in a Petri dish, were kept in an ice-box over a night or two, but were taken out some hours before the experiment. The ciliary movement of such preparations is very active at room temperature and could satisfactorily be used for experiment. Such preparations could be preserved for many days in the ice-box and used for experiments, although preparations kept in the ice-box for only one night were preferred in the present work. Only healthy tissue was used and any irregularities of structure were rejected, as the gill structures of bivalves often indicate that they have undergone injury and regenerative processes.

In the experiment, the gill piece thus prepared is allowed to crawl

in a glass tubing, which we provisionally call a "measuring tube" (fig. 1). This forces the gill piece to crawl in a straight line and prevents any deviation from the straight line, that may occur owing to any asymmetry of the preparation. The gill piece proceeds with the ventral margin in front at a quite uniform velocity as long as the conditions remain unchanged. The tube was graduated in millimetres, and the velocity of the progress of the gill piece was measured by reading the scale every minute, as a rule, at the graduation which the front margin of the gill piece was passing. The velocity of the gill piece in the measuring tube with normal sea-water was taken as 100 per cent. and the velocity of the same gill piece in the experimental solution was expressed in percentage of the normal. Any slight disturbance, mechanical, chemical or any other, interferes with the velocity and should be avoided as far as possible. The transfer of the gill piece from the normal sea-water to the experimental medium could not therefore be performed with a spatula or with any other instrument, and consequently was a matter of difficulty. To overcome this, we have devised various types of measuring tubes. The one used in the present study was as is shown in fig. 1.

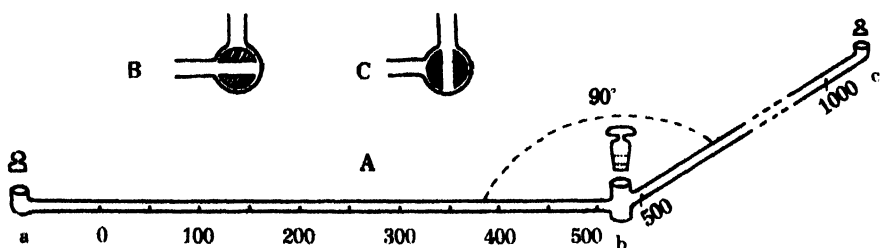


Fig. 1. A: Measuring tube of ciliary movement. The tube is graduated in millimeters, the first arm of the tube has graduations 0 to 500 and the second arm 500 to 1000. B: position of the cock allowing the entrance of the gill piece from the first arm. C: position of the cock allowing the entrance of the gill piece into the second arm.

The tube consists of two arms, joining with each other at a right angle at point *b*, provided with a well ground glass cock. The bore of the cock, about one centimetre, is equal to that of the arms of the tube, and allows the gill piece to slide smoothly in and out of the cock, otherwise the unevenness of the bottom surface of the tube would hinder smooth progress of the gill piece. In the experiment, the first arm of the tube, *ab*, contains normal sea-water and the second arm *bc* contains the experimental solution, unless otherwise mentioned. When the gill piece

enters a different medium or is transferred, the velocity of the gill piece is disturbed for a short time, but the tissue comes into equilibrium with the medium in a short time and shows a quite uniform velocity. But the velocity in the first part of the arm may not be dependable in all cases and should be discarded, as a slight change of medium conditions may affect the velocity. The first arm of the tube was graduated from 0 to 500 and the second arm from 500 to 1000. Each arm has, moreover, some distance of ungraduated part, in order that the tissue may come into equilibrium with the medium before the velocity is measured. It should be kept in mind in reading the tables, when the course of the gill piece is repeated in one of the arms, that the readings of the scale between 0 and 500 refer to the course of the gill piece in the normal medium, *i. e.*, sea-water, and that those between 500 and 1000 refer to the progress of the piece in the experimental solution, unless otherwise mentioned. The gill piece crawls on the bottom surface of the tube and any slight roughness of the bottom may interfere with the velocity, so the latter for each minute fluctuates, but it is quite uniform when averaged for a sufficiently long distance. The availability of the velocity of the gill piece crawling in the measuring tube as the measure of the ciliary activity was carefully worked out in our previous papers (NOMURA and TOMITA 1933; TOMITA 1934 *a* & *b*), and for details of the method the reader is referred thereto. The main point of modification of the method in the present study is that the tube is provided with a cock at the junction of the two arms and the gill piece is allowed to proceed without interruption through the cock from the normal medium to the experimental solution, thus minimizing the mechanical disturbance that is otherwise unavoidable. When the gill piece crawls in the first arm of the tube, the cock is open to the first arm as is shown in position B in fig. 1, and when the piece has entered the cock, the latter is gently turned and opened to the second arm, position C, so that the piece can crawl out smoothly.

The adrenaline solutions were made whenever possible immediately before using, as the freshness of the solution was an indispensable factor of the experiment. Adrenaline rapidly undergoes oxidation in solution, especially in alkaline reactions, and may give the solution a red tint when it is concentrated enough, due to the oxidation product. The original solution of adrenaline used in this study was a commercial preparation of l-adrenaline chloride (l-o-dioxyphenyl-ethanol-methylamine chloride) of the concentration of 1 part in 1000, ( $10^{-3}$ ), supplied by Sankyo Co., Tokyo.

## EXPERIMENT

Three series of experiments with adrenaline and acetyl-choline were carried out in different seasons. In the present paper, experiments with adrenaline are reported, and those with acetyl-choline will be given in our next paper which will follow shortly. The results are given in the form of tables and figures and the main points may be understood therein, but a few points will be explained briefly in the text.

*Experiment Ser. I, No. 1.* (Table 1) Adrenaline  $10^{-6}$ .

In this experiment, both the first and the second arms of the measuring tube were filled with normal sea-water at first, and the normal velocity of the gill piece was determined in three courses, the first course in the first arm, the second course in the second arm, and the third course again in the first arm. The repetition of the course was made by turn-

TABLE 1.

Experiment Ser. I, No. 1. Adrenaline  $10^{-6}$   
Temperature  $24^{\circ}.9 \sim 25^{\circ}.6$  C.

Normal sea-water						Adrenaline	
Time	Scale	Time	Scale	Time	Scale	Time	Scale
min.	mm.	min.	mm.	min.	mm.	min	mm.
0	30	20	532	0	10	13	
1	55	21	571	1		14	
2		22	601	2		15	536
3	105	23	644	3		16	567
4		24	684	4	177	17	589
5	153	25		5	219	18	608
6		26		6	257	19	631
7		27		7	296	20	651
8		28		8	335	21	676
9		29		9	369	22	694
10	303	30	905	10	403	23	714
11	336	31	942	11	438	24	733
12	365			12	479	25	752
13	397	Average 37.2		Average 39.0		26	771
14		Average 38.1				27	789
15	453					28	808
16	498					29	828
Average 29.2						30	848
						31	869
						32	890
						33	911
						34	929
						35	953
						36	974
						37	996
						Average 20.0 (54.8%)	

ing the cock of the tube or by the transfer of the gill piece from the end of the second arm to the beginning of the first arm.

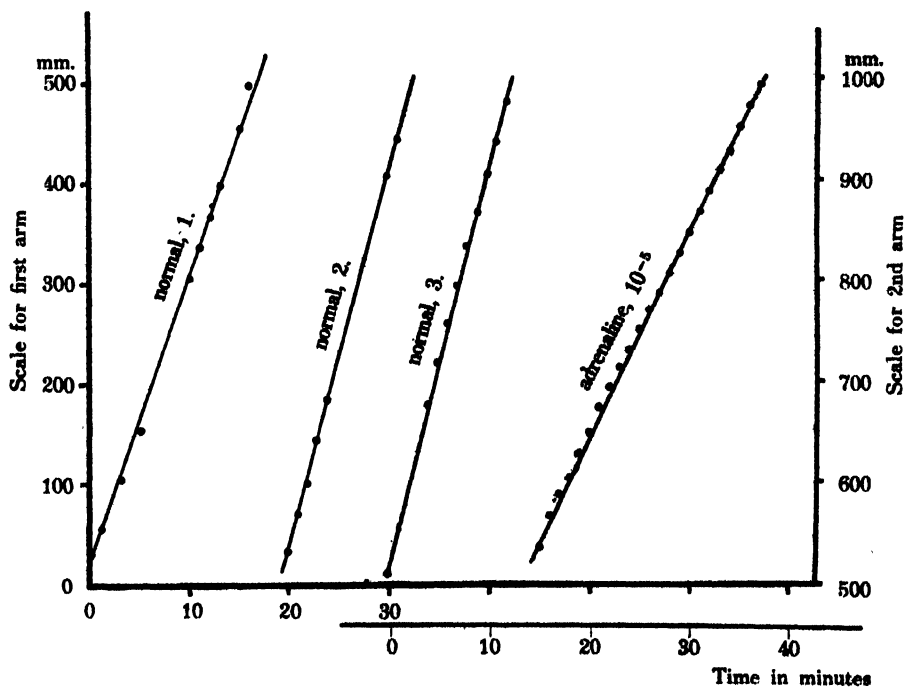


Fig. 2. Exper. Ser. I, No. 1. Adrenaline  $10^{-3}$ . The first three lines (normal, 1, 2 and 3) represent the three successive travels of the gill piece in the normal sea water, and the fourth line that in the adrenaline solution, showing distinct depressive effect. *Ordinates*: reading of the scale of the measuring tube traversed by the gill piece. *Abscissae*: experimental time. Upper figures refer to the first two courses and the lower figures to the others.

The curves obtained by plotting the readings of the scale against time are nearly parallel with each other. The first normal course shows a considerably lower velocity than the following two normal courses. This is perhaps due to the insufficient period of time allowed to enable the gill piece to come into equilibrium with the experimental temperature, after being taken out of the ice-box. The second and the third courses, however, show that the velocity of the gill piece is quite uniform. After the third course in the first arm of the tube, the sea-water in the second arm was replaced with the adrenaline solution, the concentration of which is one part of adrenaline in  $10^6$  parts normal sea-water. The gill piece was allowed to crawl in this solution and the velocity of crawl was

decreased from 39 mm/sec. to 20.9 mm/sec. (54.8%). The solution at the end of the experiment showed a slight coloration due to the oxidation product of adrenaline in the sea-water. The average velocity in the first course was rejected, and the second and third courses gave an average normal velocity of 38.1 mm/sec. The velocity in the first course in the adrenaline solution is 20.9 mm/sec. and this corresponds to 54.8% of the average normal velocity. The heavy letters in the tables are the main figures used in computation or the resulting figures. The result of this experiment clearly shows that adrenaline depresses the ciliary activity of the oyster gill, the depression amounting to 45.2% of the normal.

*Experiment Ser. I, No. 2.* (Table 2) Adrenaline  $10^{-5}$ .

The reading of the scale of the tube was begun when the gill piece passed the 10 mm. mark. The ciliary motion was very active and the

TABLE 2.

Experiment Ser. I, No. 2.

Adrenaline  $10^{-5}$ 

Temperature 26.0~25.6°C.

Normal sea-water		Adrenaline		Normal sea-water	
Time	Scale	Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	10	11		45	553
1	60	12		46	589
2	109	13	528	47	626
3	161	14	553	48	666
4	211	15	561	49	706
5	261	16	565	50	747
6	308	17	565	51	786
7	353	18		52	823
8	398	19	572	53	860
9	445	20	573	54	898
10	493			55	933
Average 48.3		Average 6.4		56	970
		22		Average 37.9	
		23	*	Recovery	
		24	732		
		25			
		26			
		—			
		32			
		33	739		
		34	*709		
		35	709		
		—			
		—			
		complete depression		* displaced	



piece traversed the whole course of the first arm in ten minutes, showing an average velocity of 48.3 mm/sec. The piece passed the cock and entered the adrenaline solution in about 11 minutes. The velocity immediately decreased and complete stoppage ensued in a few minutes (Fig. 3). When the cilia beat very feebly, the gill piece can not crawl, so complete stoppage of the locomotion of the gill piece does not always mean the complete stoppage of the ciliary beat. The piece may sometimes proceed if aided, when the ciliary beat is not too feeble. So the tube was inverted to aid the piece to move if possible, and the piece was transferred to the 732 mm. mark (see arrow in the figure), but it remained there motionless, and further displacement (see arrow in the figure) could not bring about any further progress.

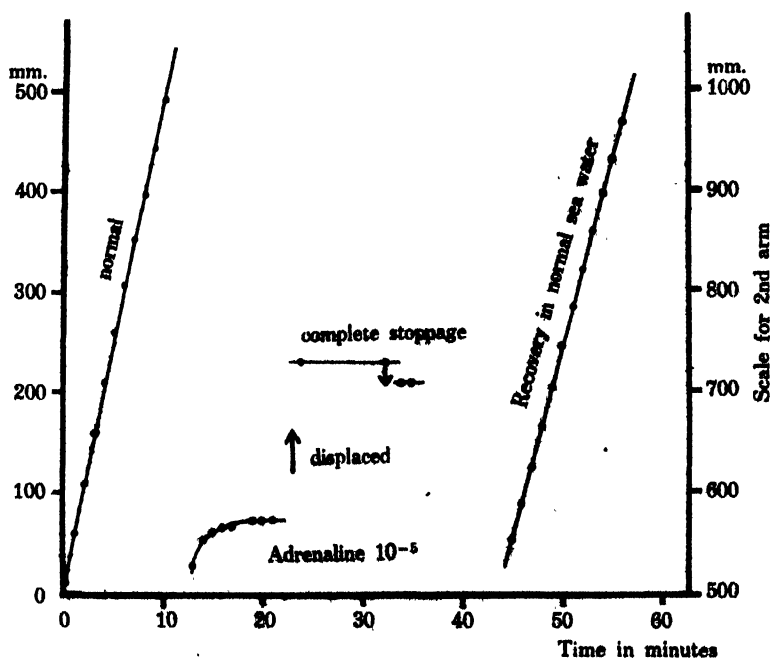


Fig. 3. Exper. Ser. I, No. 2. Adrenaline  $10^{-5}$ . Progress in the normal sea-water is represented by a straight line, that in the adrenaline solution shows prompt decrease and subsequent complete stoppage. Arrows indicate displacement of gill piece to aid possible progress by residual ciliary movement. Other explanations as in the preceding figure.

Then the adrenaline solution was replaced with fresh normal sea-water and the tissue piece recovered greatly in a few minutes. The experiment

proves, therefore, that the progress of the gill tissue is completely depressed in an adrenaline solution of a concentration of  $10^{-6}$ , and it also shows prompt and almost perfect recovery in normal sea-water.

*Experiment Ser. I, No. 3.* (Table 3) Adrenaline  $2 \times 10^{-6}$ .

In this experiment, the ciliary progress of the gill piece was completely stopped after 10 minutes in adrenaline solution, but it was resumed after displacement. Whether the stoppage was due to an obstacle in the tube, or whether recovery was due to inactivation of adrenaline in the lapse of time was tested in the next experiment. Even after recovery of the progress, the velocity was decreased to 80% of the normal. But it should be noted that the velocity was rising near the end of the experiment and exceeded the normal. The velocity at the beginning of the experiment was rather decreasing, and in the adrenaline solution decreased and fell to zero, and then rose again after being aided. Whether this is an anomaly, or recovery by acclimatization, or whether the supernormal velocity near the

TABLE 3.

Experiment Ser. I, No. 3.

Adrenaline  $2 \times 10^{-6}$

Temperature =  $26.5 \sim 27.1^\circ \text{C}$

Normal sea-water		Adrenaline			
Time	Scale	Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	23		41	*756
1	31	24		42	
2	64	25		43	771
3	93	26		44	784
4	125	27		45	794
5	150	28	506	46	798
6	176	29		47	814
7	201	30	561	48	834
8	225	31	586	49	855
9	251	32	613	50	877
10	274	33	638	51	903
11	296	34	655	52	930
12	314	35	675	53	959
13	333	36	699	54	985
14	349	37	700		
15	369	38	703	Average	17.6
16	383	39	703		(80%)
17	397	40	703		
18	413				
19	430	complete depression			
20	448				
21	471				
22	486				
Average	22.6				

\* displaced

end of the experiment is a phenomenon, such as is often the case in recovery from the effect of narcotics or drugs, remains a problem.

*Experiment Ser. I, No. 4.* (Table 4) Adrenaline  $2 \times 10^{-6}$ .

In the preceding experiment, the gill piece resumed progress when it was displaced. It is possible, therefore, that the depression of the ciliary motion may have been incomplete and feeble ciliary movement may have been going on without actual progress of the gill piece, and the progress became actual again when the piece was aided, or that the oxidation of the adrenaline gradually proceeded and the effect of the solution so decreased as to allow the tissue piece to progress. The adrenaline solution used in this experiment was the same that had been used in the preceding experiment, but the gill piece was a new one. In this experiment, we tried to test the efficiency of an adrenaline solution that had been once used and that was about one hour old after preparation at the beginning of the experiment. In normal sea-water the velocity was 47.2 mm./sec. and decreased to 37.4 mm./sec. for a few minutes, in the adrenaline solution, then the piece encountered at 20 min. the gill piece of experiment 3 that was coming back on the reverse course, and they crossed over each other. The later progress of the gill piece showed an average velocity of 32.8 mm./sec. (69.4%). This experiment shows, therefore, that the adrenaline solution in the concentration of  $2 \times 10^{-6}$  is sufficiently effective even after being once used to produce about a 30%

TABLE 4.

Experiment Ser. I, No. 4.  
Temp. = 27° 1 C.

Adrenaline  $2 \times 10^{-6}$

Normal sea-water		Adrenaline			
Time	Scale	Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	11		22	830
1	—	12		23	866
2	96	13		24	900
3	149	14	537	25	931
4	198	15	575	26	962
5	249	16	611	27	994
6	296	17	647	Average 32.8 (69.4%)	
7	340	18	686		
8	387	19	724		
9	427				
10	472	Average 37.4			
Average 47.2		* see text.			

decrease in velocity. The complete stoppage in the preceding experiment might be, therefore, a transient phenomenon.

*Experiment Ser. I, No. 5.* (Table 5) Adrenaline  $2 \times 10^{-7}$ .

The velocity in the normal sea-water is quite uniform at the beginning and at the end of the course, averaging 38.9 mm./sec., and the plottings of the curve fall into a nearly straight line. In the adrenaline solution, the velocity was decreased slightly at first, then more decidedly near the middle of the course, as is represented by the two lines that join each other near the middle of the course. The average velocity in the adrenaline solution is 30.1 mm./sec., and corresponds to 77.3% of the normal. The depressing effect of adrenaline is quite evident and is far beyond the limit of experimental errors.

*Experiment Ser. I, No. 6.* (Table 6) Adrenaline  $2 \times 10^{-8}$ .

In the normal sea-water the velocity tended somewhat to decrease, but on entering the adrenaline solution the velocity gradually increased up to 48 mm./sec. (Table 6, second column). The age of the solution after preparation was not recorded, and whether the increase in the adrenaline solution was the temporary effect of low concentration of the substance, as may often be the case with other substances which are characteristically depressive, or whether the accelerative effect here observed is the characteristic action of adrenaline remains to be determined in further experiments. The gill preparation, therefore, was returned to the beginning of the first arm of the measuring tube after it had finished the course in the adrenaline solution in the second arm of the tube. In handling, the piece shrank somewhat and remained quite motionless for a moment, but then regained movement and proceeded to the second course in the normal sea-water in the first arm of the tube.

TABLE 5.

Experiment Ser. I, No. 5.

Adrenaline  $2 \times 10^{-7}$ 

Temperature—26°.9–26°C

Normal sea-water		Adrenaline	
Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	16	
1	38	17	508
2	75	18	538
3	118	19	572
4	158	20	608
5	198	21	638
6	241	22	671
7	279	23	700
8	317	24	731
9	355	25	766
10	393	26	794
11	429	27	822
12	467	28	850
		29	880
Average	38.9	30	909
		31	936
		32	962
		33	990
		Average	30.1 (77.3%)

The velocity in this course was 37.6 mm./sec.. The second arm of the tube was filled with a freshly made adrenaline solution, and the gill piece

TABLE 6.

Experiment Ser. I, No. 6.  
Temperature 28°.9~28°.4 C.

Adrenaline  $2 \times 10^{-8}$ .

Normal sea-water		Adrenaline		Normal sea-water		Adrenaline	
Time	Scale	Time	Scale	Time	Scale	Time	Scale
min.	mm.	min.	mm.	min.	mm.	min.	mm.
0	0	15		30		55	
1	45	16		31		56	530
2	89	17	529	33		57	563
3	132	18	567	34		58	597
4	173	19	607	35		59	633
5	212	20	646	36		60	667
6	251	21	684	37	25	61	698
7	290	22	727	38	60	62	732
8	330	23	772	39	96	63	767
9	369	24	821	40	135	64	802
10	406	25	869	41	175	65	835
11	445	26	914	42	215	66	869
12	481	27	962	43	255	67	904
13		28		44	296	68	
14		29		45	333	69	
				46	369		
				47	403		
				48	440		
				49	477		
				50			
Average 40.0 (100%)*		Average 43.3 (108.2%)*		Average 37.6 (100%)**		Average 34.0 (85.0%)* (90.4%)**	
↑		↑		↑		↑	
88.8(100%)*				88.4(88.8%)*			

in this solution showed an average velocity of 34.0 mm./sec. (90.4%). The figures in Table 6 marked with the same number of asterisks correspond to each other respectively. For instance, the average velocity 34.0 mm./sec. (90.4%) in the fourth column of the table marked with two asterisks (\*\*) refers to the average in the third column, 37.6 mm./sec., as the normal (100%). The accelerative effect observed in the second column of the table (108.2%) seems, therefore, to be an experimental error or non-specific action of adrenaline, judged from the second trial given in the third and fourth columns of the table and from other subsequent experiments.

Experiment Ser. I, No. 7. (Table 7). Adrenaline  $4 \times 10^{-8}$ .

\* The velocity in the normal sea-water was 41.7 mm./sec. on an average,

and that in the adrenaline solution was 38.4 mm./sec. (92.0%). The gill piece was allowed to make the reverse course in the adrenaline solution (note that the reading of the scale is decreasing), and the speed was found

TABLE 7.

Experiment Ser. I, No. 7.  
Temperature = 26°.7–26°C.

Adrenaline  $4 \times 10^{-6}$

Normal sea-water		Adrenaline		Adrenaline		Normal sea-water	
Time	Scale	Time	Scale	Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	100	13		30	Reverse	41	
1	151	14		31	course	42	478
2	196	15	525	32		43	—
3	242	16	563	33	747	44	403
4	284	17	602	34	708	45	369
5	322	18	639	35	673	46	333
6	358	19	676	36	637	47	298
7		20	714	37	597	48	—
8	434	21	752	38	556	49	226
9		22	790	39	516	50	193
10		23	829	40			
11		24	868				
12		25	909				
		26	945				
		27	986				
		28					
		29					
Average 41.7 (100%)		Average 38.4 (92.0%)		Average 38.5 (92.3%)		Average 35.6 (85.3%)	

to be almost the same as in the preceding course, *i. e.*, 92.3%. But the piece on entering the first arm and traversing the normal sea-water, further decreased in velocity down to 85.3% of the original normal velocity. This does not seem to prove that the effect of the adrenaline solution is accelerative, but, on the contrary, the decrease in the normal sea-water probably was the after-effect of the adrenaline solution, in which the gill piece had been exposed for about half an hour. A wash-out effect (A. J. CLARK, p. 193) is a similar phenomenon and is referred to in a subsequent page. This experiment also seems to have proved the inhibitory effect of adrenaline upon the ciliary activity.

*Experiment Ser. II, No. 10.* (Table 8) Adrenaline  $2 \times 10^{-6}$ .

The other experiments of this series, having been devoted to the effect of acetyl-choline and eserine, are omitted in this paper. In this experiment with adrenaline, the velocity at first was about 30 mm./sec. and 38 mm./sec. at the end of the course, averaging about 34.5 mm./sec. On entering the

TABLE 8.

Experiment Ser. II, No. 10. Adrenaline  $2 \times 10^{-6}$   
Temperature =  $21^{\circ}\text{C}$ .

Normal sea-water		Adrenaline	
Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	15	
1	30	16	510
2	62	17	549
3	94	18	582
4	126	19	617
5	158	20	647
6	192	21	677
7	226	22	703
8	263	23	727
9	301	24	751
10	339	25	773
11	372	26	793
12	410	27	—
13	446	28	836
14	484	29	—
Average	34.5	30	878
		31	899
		32	920
		33	941
		34	962
		35	983
		Average	24.8 (71.8%)

adrenaline solution, the piece showed 39 mm./sec. but the velocity promptly decreased, and in the second minute in the adrenaline solution the velocity fell to 33 mm./sec., and continued falling down to 21 mm./sec. for the last six minutes. The average for the course in the adrenaline solution is 24.8 mm/sec., which amounts to 71.8% of the normal.

The inhibitory effect of adrenaline was clearly proved.

*Experiment Ser. III, No. 1.*  
(Table 9 a & b) Adrenaline  $10^{-10}$ .

This series of experiments was mostly devoted to the study of acetyl-choline. The present experiment with adrenaline was

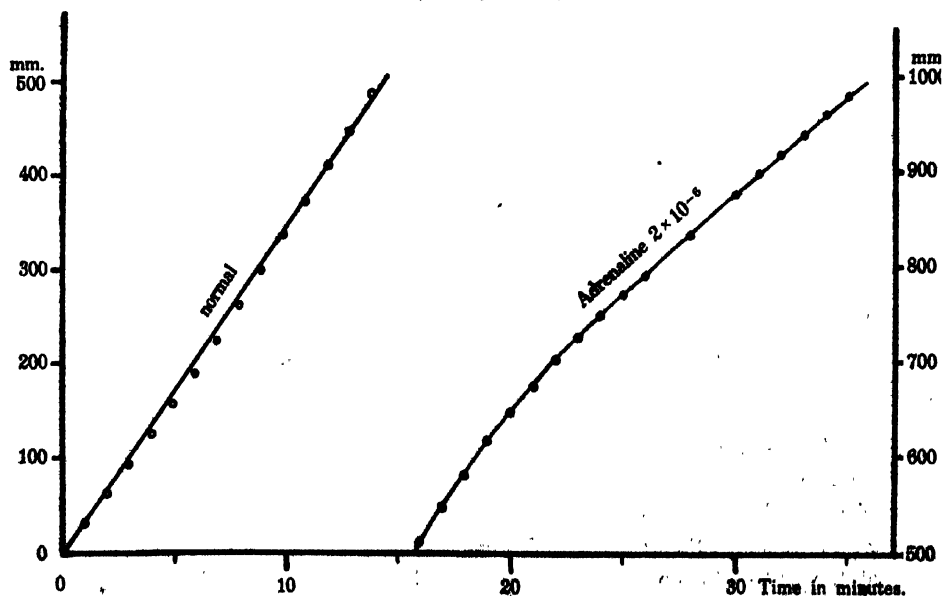


Fig. 4. Exper. Ser. II, No. 10. Adrenaline  $2 \times 10^{-6}$ . Gradual decrease in velocity and subsequent uniform velocity in equilibrium with the medium. Depression: 28.2%.

TABLE 9, a.

Experiment Ser. III, No. 1.

Adrenaline  $10^{-10}$ Temperature =  $24^{\circ}.7 \sim 25^{\circ}.6$  C.

Normal sea-water		Normal sea-water		Normal sea-water		Adrenaline $10^{-10}$	
Time	Scale	Time	Scale	Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	17		0	0	14	
1	32	18	530	1	38	15	517
2	66	19	562	2	78	16	558
3	102	20	596	3	120	17	599
4	136	21	633	4		18	
5		22	673	5	201	19	682
6	209	23	710	6	240	20	722
7	244	24	741	7	280	21	759
8		25	781	8	318	22	796
9	312	26	819	9	353	23	829
10	347	27	859	10	389	24	866
11	377	28	895	11	428	25	905
12	408	29	934	12	468	26	945
13	444	30	976	13		27	981
14	478						
15		Average	37.1	Average	39.0	Average	38.6
16					(100%)		(98.9%)

Average 34.1

TABLE 9, b.

Experiment Ser. III, No. 1, (contin.)

Adrenaline  $10^{-10}$ Temperature  $24^{\circ}.7 \sim 26^{\circ}.6$  C.

Adrenaline		Adrenaline		Normal sea-water		Normal sea-water	
Time	Scale	Time	Scale	Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
30	547	45	531	0	500	18	568
31	587	46	571	1	524	19	609
32	625	47	611	2	548	20	648
33		48	651	3	572	21	688
34	703	49	689	4	601	22	727
35	741	50	728	5	630	23	768
36	778	51	766	6	659	24	809
37	818	52	805	7	688	25	849
38	855			8	721	26	888
39	893	Average	39.1	9	754	27	928
40	930		(100.2%)	10	789	28	965
41	968			11	823		
				12	860		
				13	898		
				14	934		
				15	971		
Average	38.2			Average	31.4	Average	39.7
	(88.0%)				(80.5%)		(101.7%)
		ca. 1 hour in adrenaline solution					



begun within two hours after the gill preparations were taken out of the ice-box, in which they had been preserved. So the velocity of the gill piece was low at first, 34.1 mm./sec., but gradually increased in the second trip (second column of Table 9, a) to 37.1 mm. on an average, and then the same gill piece was returned to the beginning of the first arm of the measuring tube after an interval and the normal velocity was again measured. It rose to 39.0 mm./sec. and seemed to remain uniform, so this velocity was taken as the normal (100%) and the subsequent course in the second arm of the tube with the adrenaline solution was measured. As the concentration was very low, the effect was very slight or nearly approached the limit of errors (98.9%). Then the gill piece was returned to the starting point of the second arm, and was allowed to repeat the trip twice in the adrenaline solution (first and second columns of the Table 9, b). The effect was not distinct. After this the piece was allowed to remain in the adrenaline solution for about one hour, and the tissue somewhat expanded. On transfer to the normal sea-water, however, the tissue shrank and remained motionless for a short time, but recovered and the velocity progressively rose to 37 or 38 mm./sec. near the end of the course in sea-water (averaging 31.4 mm./sec.; 80.5%) (Table 9, b, third column) and the recovery was perfect and rather supernormal activity (average 39.7

mm./sec.; 101.7%) was observable in the second course in the normal sea-water. Probably this concentration,  $10^{-10}$ , may be the limit of the effective concentration of adrenaline on the oyster gill cilia, as far as the present method is concerned. The temporary accelerative tendency ought not be considered as the specific effect of the drug.

*Experiment Ser. III, No. 2.*  
(Table 10) Adrenaline  $10^{-7}$ .

Although the average is 43.9 mm./sec., the velocity was rising in the sea-water, and the gill piece on entering the adrenaline solution showed supernormal

TABLE 10.

Experiment Series III, No. 2.

Adrenaline  $10^{-7}$ 

Temperature 26°C.

Normal sea-water		Adrenaline	
Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	12	
1	40	13	511
2	81	14	564
3	122	15	613
4	164	16	660
5	206	17	709
6	253	18	757
7	297	19	804
8	343	20	849
9	391	21	894
10	438	22	940
11	483	23	985
Average	43.9	Average	47.4 (107.8%)

activity (even 53 mm. per sec.), but excitation seemed to be abolished, and the velocity near the end of the course in the second arm of the measuring tube approached that in the latter part of the first arm, while the average velocity in the adrenaline solution, 47.4 mm. per sec. (107.9%), did not seem to prove the definitive accelerative action of adrenaline. The solution was, moreover, three hours old after preparation. The age probably weakened the inhibitory action and the experimental error in measuring the normal velocity in insufficient duration brought about the apparently accelerative effect. This was shown clearly in the next experiment, where a fresh solution was used and the effect was distinct.

*Experiment Ser. III, No. 3.*

(Table 11) Adrenaline  $10^{-7}$ .

The results of the preceding experiment, No. 2, where the age of the solution seemed to have interfered with the action of the solution, was doubtful and re-examination of the effect of the concentration of  $10^{-7}$  was made with a fresh solution in this experiment. Here the effect is quite evident, and the inhibition is 16.6%. As is seen from the curve, the inhibition was progressive at about the first third of the course and the velocity remained almost uniform for the rest of the course.

*Experiment Ser. III, No. 4.*

(Table 12) Adrenaline  $10^{-8}$ .

The inhibition in this experiment is less than in the preceding one, as would be expected, and approaches the limit of experimental errors. But the experiment proceeded quite satisfactorily and uniformly, and the effect seems not to be an incidental experimental error (Fig. 5). The adrenaline solution was quite fresh.

*Experiment Ser. III, no. 5. (Table 13) Adrenaline  $10^{-10}$ .*

The average velocity in the normal sea-water is 42.1 mm./sec., and the same in the adrenaline solution is 40.9 mm/sec. (97.1%). Although the inhibition in this experiment is small and is nearly an experimental error.

TABLE 11.

Experiment Ser. III, No. 3.

Adrenaline  $10^{-7}$

Temperature  $26^{\circ}.5-27^{\circ}$  C.

Normal sea-water		Adrenaline	
Time	Scale	Time	Scale
<i>min.</i>	<i>mm.</i>	<i>min.</i>	<i>mm.</i>
0	0	12	
1	42	13	512
2	84	14	556
3	126	15	599
4	171	16	639
5	215	17	676
6	259	18	713
7	303	19	
8	347	20	782
9	387	21	816
10	429	22	847
11	474	23	
		24	915
Average	43.0	25	947
		26	979
		Average	85.5 (53.4%)

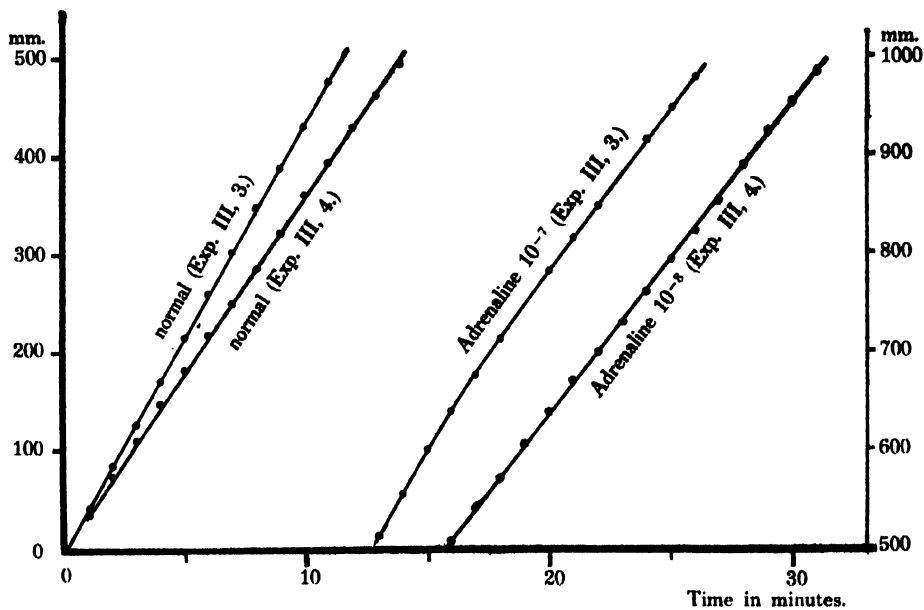


Fig. 5. Exper. Ser. III, No. 3. Adrenaline  $10^{-7}$ . Exper. Ser. III, No. 4. Adrenaline  $10^{-8}$ . Graph showing the relative effect of concentrations. Depression in  $10^{-7}$  adrenaline solution is 16.6%, and in  $10^{-8}$  solution is 8.9%. Compare also fig. 4.

TABLE 12.

Experiment Ser. III, No. 4.

Adrenaline  $10^{-8}$ Temperature =  $25^{\circ}.3 \sim 26^{\circ}.5$  C.

Normal sea-water		Adrenaline	
Time	Scale	Time	Scale
min.	mm.	min.	mm.
0	0	15	
1	37	16	508
2	74	17	541
3	111	18	574
4	148	19	606
5	183	20	639
6	217	21	671
7	250	22	701
8	285	23	731
9	321	24	761
10	359	25	793
11	392	26	823
12	428	27	855
13	460	28	891
14	491	29	924
Average		30	956
		31	987
		Average 81.9 (81.1%)	

TABLE 13.

Experiment Ser. III, No. 5.

Adrenaline  $10^{-8}$ Temperature  $26^{\circ}.5$  C.

Normal sea-water		Adrenaline	
Time	Scale	Time	Scale
min.	mm.	min.	mm.
0	0	13	
1	43	14	521
2	88	15	565
3	133	16	609
4	177	17	652
5	219	18	695
6	260	19	736
7	299	20	778
8	339	21	818
9	381	22	857
10	422	23	896
11	464	24	933
12		25	971
Average 42.1		Average 49.0 (87.1%)	

it seems to me to be significant that the experiments (Nos. 3, 4 & 5) with the decreasing concentrations ( $10^{-7}$ ;  $10^{-8}$ ;  $10^{-9}$ ) show also the regularly decreasing magnitudes of inhibition. Moreover, the next lower concentration,  $10^{-10}$ , shows also the lesser inhibition (Exper. Ser. III, No. 1, Table 9, a & b). All the foregoing experiments seem to have proved that adrenaline at the concentrations as far as examined,  $10^{-5}$  to  $10^{-10}$ , inhibits the ciliary activity of the ciliated epithelium of the gill of the oyster, and no decisive evidence of definitive accelerative effect was observed so far as the present experiments are concerned.

## COMPARISON AND DISCUSSION

The results of the experiments are summarized in Table 14 and Fig. 6. All the experiments except Exp. Ser. III, No. 2 ( $10^{-7}$ ) and Ser. I, No. 6 (first determination,  $2 \times 10^{-8}$ ) show clearly the depressive effect of adrenaline on the ciliary movement. The exceptional effect observed in these experiments just mentioned may be explained by the conditions of the experiments described under the headings of the respective experiments.

TABLE 14.

Concentration of adrenaline	No. of experiments	Per cent. velocity in adrenaline solution
$10^{-5}$	Ser. I, No. 1	54.8
"	Ser. I, No. 2	0.—
$4 \times 10^{-6}$	Ser. I, No. 7	92.3
$2 \times 10^{-6}$	Ser. I, No. 3	0—80
" "	Ser. I, No. 4	69.4
" "	Ser. II, No. 10	71.8
$2 \times 10^{-7}$	Ser. I, No. 5	77.3
$10^{-7}$	Ser. III, No. 2	107.9
"	Ser. III, No. 3	83.4
$2 \times 10^{-8}$	Ser. I, No. 6	108.2; 85.0; 90.4; 96.3
$10^{-8}$	Ser. III, No. 4	91.1
$10^{-9}$	Ser. III, No. 5	97.1
$10^{-10}$	Ser. III, No. 1	98.9; 98.0; 100.2

Diphasic action of adrenaline has sometimes been described by some authors, and the accelerative effect observed in the exceptional cases just mentioned may also be reconciled with the normal depressive action by considering the diphasic action, and the depressive effect ought to be

considered as the specific effect of adrenaline upon the ciliary activity of the epithelium of the oyster gill.

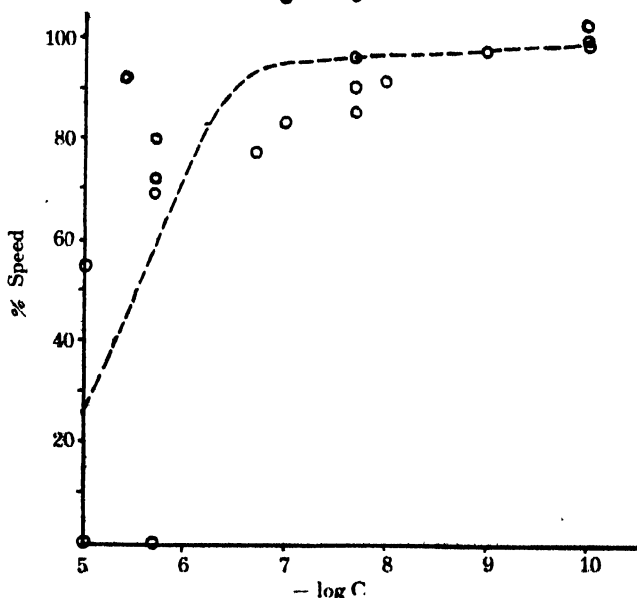


Fig. 6. Graph showing the relation between the crawling speed of the gill piece and the concentration of adrenaline solutions. *Ordinates*: crawling speed in the adrenaline solution in percentage of the normal. *Abscissae*: -log concentration adrenaline in sea-water.

Transient effects of adrenaline and of many other drugs have been observed by previous investigators. MEYER (1908) first noted this effect in the cases of adrenaline acting on arterial strips. NEUKIRCH (1912) noted that a large dose of pilocarpine produced an initial stimulation of the isolated gut, that this stimulation passed off after prolonged exposure to the drug, but that wash-out caused a second powerful stimulation (A. J. CLARK, p. 193). In our experiment (table 9, a & b) a similar effect is also observable. The very dilute concentration ( $10^{-10}$ ) of adrenaline depressed the ciliary activity slightly at first, then the effect passed off and normal velocity re-appeared. After about one hour in the adrenaline solution, the gill piece again showed the depressed velocity (80.5%) on transfer to the normal sea-water. This second depression was also followed by the normal velocity (or supernormal about the limit of experimental errors).

STRAUB put forward the so-called potential theory of the actions of drugs: *i. e.* that the action of drugs depends on a potential gradient between the concentrations of the drug outside and inside the cells. This theory found many supporters, but FRITZ (1928) maintains that the adrenaline recovery of isolated gut was due chiefly to the breakdown of the drug, and that the effects frequently recorded on wash-out were due to temperature alterations. He concluded that potential effects were chiefly a summation of experimental errors (A. J. CLARK, p. 193). Potential theory seems to be able to explain the recovery in the drug solution, but not to be able to explain the temporary excitatory effect of drugs of depressive action in dilute concentrations or on wash-out. The ARNDT-SCHULZ law of the action of drugs may be consulted in this connection. It states that "Weak stimuli excite, medium stimuli partially inhibit and strong stimuli produce complete inhibition." (A. J. CLARK, p. 195). As to the transient and polyphasic actions, opinions are divergent, but the fact may be mentioned in this connection that the duration of adrenaline action is at most a few hours in man, dilates at first the pupils and then constricts them to pin-points (McCLENDON 1937). The exceptional accelerative case of the action of adrenaline observed in the present work may be understood by taking into account such polyphasic action. BACQ suggests that the differences between sympathin I and sympathin E could be explained by assuming sympathin E to be identical with a slightly oxidized adrenaline. He writes, moreover, that "*Si l'on pouvait prouver que l'adrénaline légèrement oxydée perd ses propriétés inhibitrices plus rapidement que ses propriétés excitatrices nous aurions la possibilité, actuellement exclue, d'opposer à la théorie de CANNON et ROSENBLÜTH (1933) une interprétation rationnelle des faits.*" This hypothesis was denied by BLASCHKO and SCHLOSSMANN (1936). From this point of view, it becomes quite necessary to study the effect of drugs in wide ranges of concentrations, to determine the specific action of the drugs. The statement of ARNDT-SCHULZ is really exemplified in our experiment with acetylcholine, the results of which will be published immediately. In the present study, the effect of adrenaline was examined in concentrations of  $10^{-8}$  to  $10^{-10}$ . This range seems to be sufficient, judging from our experiments and the work of many previous workers, although some few investigators examined more dilute concentrations. In the higher concentrations, the inhibitory effect is evident, and the lowest concentration shows only the slightest degree of inhibition, that may approach the limit of experimental errors, or almost no action. The molecular weight of adrenaline is 183.16, and the concentration of  $10^{-10}$

contains about  $3.3 \times 10^8$  molecules per cmm. or 0.33 molecules per  $\mu^2$ . One molecule of adrenaline probably covers an area between 20 and 50 sq. Å, and if the latter figure be taken, then the number of molecules required to cover one heart-cell of the frog is of the order of  $10^{10}$  molecules. The dimension of the frog heart ventricle cell is  $131 \mu \times 9 \mu$  and the number of the cells in a cmm. is  $0.33 \times 10^6$ , their total surface in a sq. cm. being 6. As to the dimension of the oyster gill cells, we have no concrete data, but the cells are relatively small as is usual in molluscs and the figures in allied groups have been given as follow: the cells of the gill filament of *Crepidula plana*,  $6 \times 11$  in micra; cells of ganglion cerebrale of *Helix ericetorum*,  $51 \times 33$  to  $9 \times 9$  in micra, maximum and minimum respectively (Tabulae biol. IV). The cells of the oyster gill may be assumed to be about of the same size. If the action of a drug depends upon the accumulation of the drug at the cell surface, as is maintained by some authors, the smaller cells in a given volume will require a larger number of molecules per unit volume. The tolerance of the oyster cells for narcotics and chemicals is rather high as judged from our experience with the oyster gill (NOMURA 1928, NOMURA and IMAI 1936), so the minimum effective concentration of adrenaline would also be expected to be rather high, and more dilute concentrations of adrenaline than those we have examined probably need not be tested.

The minimum concentrations of adrenaline that produce demonstrable actions in a rabbit's intestine (inhibition) is  $5 \times 10^{-9}$  molar, in a rabbit's ear (vasoconstriction)  $5 \times 10^{-10}$  molar, in a rat's uterus (inhibition)  $5 \times 10^{-10}$  molar (A. J. CLARK, table VI). The molecular weight of adrenaline being 183.16, the minimum concentration we have examined corresponds to  $\frac{1000}{183.16} \times 10^{-10}$  molar, i. e. approximately  $5 \times 10^{-10}$  molar. ALGREN (1926) and EULER (1930) report that adrenaline influences the oxygen consumption of tissues at a concentration of 1 part in  $10^{14}$ , but others failed to find the effect in such dilutions: of example, KISCH and LEIBWITZ (1930) found that the limit of demonstrable activity was 1 in  $10^4$ . KRAWKOW (1923) on the contrary claims that adrenaline at a dilution of 1 in  $10^{16}$  produced constriction in a perfused rabbit's ear, but KOPP and MANCKE (1930) found that the limit of demonstrable activity was 1 in  $10^5$ . Extreme figures were given by SCHLOSSMANN (1927) who observed that adrenaline at a dilution of  $10^{-18}$  produced an effect on a frog's heart partly poisoned with aconitine. A. J. CLARK warns against such extreme figures of low dilutions, but he states at the same time — "Indeed their ex-

pression involves the use of figures of unfamiliar order of magnitude. If however, these figures are expressed as the number of molecules present, then the results show that there is no necessity to assume special laws of chemistry or physics in order to explain the effects observed" (A. J. CLARK, pp. 26-27). Although the limit concentrations of the demonstrable effect differ greatly, according to the authors, as have been mentioned above, the quality of effects do not differ according to the concentrations of adrenaline. It is inhibitory or excitatory according to the nature of the tissues acted upon, or to the nature of innervation; increased contraction in the sphincter of the pylorus and of the ileocaecal valve, spleen, vagina, uterus, vas deferens, retractor penis, or inhibition in intestine, stomach, oesophagus, gall-bladder, urinary bladder (SCHÄFER 1916). LANGLEY's statement may here be cited that although the rule is perhaps not entirely without exception, the result of suprarenal injection is, as a general principle, identical with that of stimulating the endings of the sympathetic nerves throughout the body (SCHÄFER 1916). In the higher animals or their tissues, the innervation would complicate the analysis of the action of the drug. The advantage of the material of our experiment is that the ciliated epithelium of the gill of the oyster under investigation is devoid of nervous connection, and can also be observed in direct contact with the medium of known concentrations of the drug. The changes of the ciliary activity can, therefore, be directly correlated either to the effect of the external conditions or to the drug, and nervous control can be put out of consideration.

Nervous control of ciliary movement, however, has long been debated by many authors, but the recent tendency among authors is to recognise two types of ciliated organs: the first type is found in the majority of these organs, and is characteristic of those which function continuously during the life-time of the animal. In these the beat is uninterrupted and apparently automatic, and in many organs of this type, independent of the presence of the other tissues of the body, *e.g.* ciliated cells of the *Mytilus* gill. In the second type, which is less common but occurs widely in the animal kingdom, the beat is intermittent or occasional and is controlled by the nervous system, as CARTER claims to have found evidence for it in the ciliated cell, velar cilia of *Nudibranch veliger* (CARTER, 1926; GRAY, 1920).

We may pause here for a moment to cite some recent papers on this problem, leaving out old literature. MERTON (1923) found that electrical stimulation of nerves leading to the ciliated epithelium of the snail's lip produced activation of previously quiescent cilia. McDONALD, LEISURE,



and LENNEMAN (1928) found that electrical and chemical stimulations of the sympathetic fibres which innervate the ciliated epithelial lining of the frog's pharynx cause the cilia to beat rapidly, and that stimulation of parasympathetic fibres causes the cilia to beat slowly. SEO (1931) definitely proved that the palate epithelium of the frog is innervated by nerve fibres from Nervus facialis, N. trigeminus and N. glossopharyngeus, and that the ciliary movement of the epithelium is accelerated by stimulating these fibres, although he at the same time does not deny neuroid transmission of the ciliary activity. FRIEDRICH (1933) also reports the nervous regulation of ciliary movement in the locomotion of Nemertini. It is highly interesting to know that SHMAGUENA (1935) more recently reports inhibitory effect of atropin, adrenaline and sympathetic nerves and accelerative effect of vagus. SCHMAGINA (1936) observed the effect of atropin, adrenaline and electrical stimulation on the ciliated epithelium of the oesophagus of the frog and on the trachea of the cat and the dog. The result showed that besides automacy of the ciliary movement there is also a nervous inhibition. All these results just mentioned furnish examples of nervous regulation of ciliary movement, and they are in agreement with each other. If we come back to materials of more intimate relation to our material, we can not find any evidence of nervous control of ciliary movement.

According to FREIDENFELT (1897) "Der Nervus brachialis (das Osphradialganglion) soll ein selbständiges Zentrum für die Kiemen bilden, in dem er Ganglienzellen enthält, die Neuriten nach den Kiemenmuskeln senden, und in dem auch sensible Nervenfasern enden. Diese Stämmen teils von primären Sinneszellen des Osphradiums, teils von Zellen mit freien Nervenendigungen im Epithel der Kiemen" (HANSTRÖM 1928, p. 206). SPITTSTÖSSER studied the nervous system of *Anodonta cellensis* and described the finer branches of nerves in detail throughout the body, but did not give any description of the nerve fibres in connection with the branchial epithelium. DAKIN (1909) describes the nerve in the principal and ordinary gill filaments of *Pecten*, as running along the interlamellar margin of the filament between the epithelium and chitinous frame work, but did not make out the distribution of finer branches and their terminations.

SETNA too studied the neuro-muscular mechanism of the gill of *Pecten*, but he did not touch the problem of nervous control of the ciliated cells. He cut the subsidiary branchial nerve, but could not find any evidence of reversal of ciliary current either on the gill or on the palps. Mechanical stimulation also did not alter the direction of the ciliary stroke. No further information of the innervation or nervous control of the ciliated

cells of the gill can be found in his paper.

LUCAS recently took up the problem again and examined the innervation of the ciliated gill epithelium of Lamellibranchs (*Mytilus edulis*, *Modiolus modiolus*, *Amblema costata*, and *Megalonaias gigantea*), but could not find any morphological basis for supporting nervous control of ciliary movement, nor could he observe any response of the ciliated cells to electrical stimulation through the branchial nerve and cerebro-visceral connective. He used for stimulation a current from a Harvard induction coil with one dry cell. This may be insufficient in strength. Indeed he observed that "no muscular contraction in any portion of the animal was produced by stimulation." (LUCAS 1931 a). The failure of response may be due to large *chronaxie* of the gill tissue of *Mytilus*, as is often the case when excitable tissues with large *chronaxie* are stimulated with an alternative current of a rather low voltage and large frequency. In stimulating such tissues, the theory of *chronaxie* (LAPICQUE 1926) should be borne in mind. LUCAS's experiment thus leaves some doubt open for further investigation, but his morphological study led him to conclude that the branchial nerve is sensory and that none of the fibres enter the gill (LUCAS 1931 b). He could not find any nervous elements in connection with the ciliated cells of the gill, although his technique revealed nervous elements in other parts of the body.

After all, we have to conclude from the present status of our knowledge that the ciliary movement observed in our experiments is not controlled by the nervous system, and is acted upon directly by the drug.

Some data on the velocity of locomotion or current caused by cilia or flagella may be compared for convenience. Spermatozoa of *Bos taurus* move at a speed of  $67 \mu/\text{sec.}$ , that of man at  $310 \mu/\text{sec.}$ , *Paramoecium caudatum* swims at  $1300 \mu/\text{sec.}$ , and *Halteria sultans* at  $2300 \mu/\text{sec.}$  Lycopodium on the tracheal epithelium of a living dog was carried down by ciliary current at a velocity of 60 to  $330 \mu/\text{sec.}$  (BÜTTNER-WOBST, Tabulae biol. IV: 484). The average velocity of the gill piece in normal sea-water observed in the present experiment sometimes exceeds 4 cm./min., i. e.,  $660 \mu/\text{sec.}$ , and is of about the same order of magnitude as the values just cited, and is comparable with them.

The distribution and action of hormones in invertebrates have been investigated by many workers, and an enormous literature is being accumulated. The presence of adrenaline or chromaffine cells, that have been believed to contain adrenaline or allied substance, has been described by many authors in invertebrates: Gastropods, *Hirudo*, *Aphrodite* and

*Eunice*, *Lumbricus herculeus* (ROGERS 1927; GASKELL 1914, 1919). UNGER recently observed that excitation of certain nerves of Cephalopods discharges a substance which can be characterized by its pharmacological properties: positive inotrope action on the isolated heart, stimulation of contraction of perfused stomach and inhibition of these effects by cocaine. The presence of a hormone in the crustacean eye stalk is a well known fact nowadays, and it has been claimed that adrenaline is present even in *Paramoecium* (BAYER and WENSE 1936). As to the effect of hormones and drugs, usually found and studied in vertebrates, on the invertebrate animals the results are diverse and discrepancies are not infrequent. TAKATSUKI (1933) reports that the heart of the oyster, when affected by adrenaline, always accelerates its pulsation and increases the tone of its muscle. The minimum effective concentration of the drug varies in different individuals, but it is approximately  $1.7 \times 10^{-6}$ . This is by far a higher concentration than the minimum we have examined. TSUNODA maintains that the action of adrenaline on the heart of the oyster is always accelerative on the auricle, and is inhibitory on the ventricle, while TAKATSUKI claims that "There is no great difference between the effects of adrenaline on the ventricle and the auricle." (TAKATSUKI 1933). Shortly after their work, SUZUKI (1934) described the presence of ganglion cells in the auricle and ventricle of the oyster heart. This probably makes the problem more complicated. MEDVEDEVA in his studies on the effects of adrenaline and insuline on various invertebrates (*Aurelia aurita*, *Anodonta*, *Potamobius astacus*, and silkworm) found that adrenaline did not cause hyperglycaemia in *Aurelia* and *Anodonta*, while the effect depends on the developmental stages in the silkworm. WOLF-HEIDEGGER (1935) injected adrenaline and insuline solutions into the body of the pulmonate, *Helix pomatia*, but could not observe any definite effect upon the blood sugar level of the animal. ROCHE and DUMAZERT (1936) also report the absence of effects of adrenaline as well as of insuline and exercise on the glucose level of the haemolymph of *Cancer pagurus*. Large concentrations of adrenaline (1:100) could not affect the slow muscle of *Pecten opercularis*, (BAYLISS, BOYLAND, and RITCHIE 1930), and a similar absence of the effect of adrenaline was observed also in the heart of an ascidian, *Ciona intestinalis* (BACQ 1934 b). The negative results of the experiments with adrenaline on invertebrate animals led these authors to suppose that some mechanism for regulating the blood sugar level, or muscular activity and other processes in lower animals seems to exist, which is entirely different from the mechanism in higher animals. On the other hand, many authors report positive results of adrenaline.

effects in invertebrate animals. HOGBEN and HOBSON (1924) observed that adrenaline and epinine in concentrations of 1:500,000 evoked a very characteristic response: a marked increase in tone with the final arrest of the heart in systole is well seen, with very dilute solutions marked acceleration of the slowly beating heart was found to follow perfusion with adrenaline or epinine, but the acceleration was in general obscured by the immediate tonic contraction of the ventricle. BACQ (1934 a) reports recently that adrenaline in concentrations of  $1 \cdot 10^{-7}$  to  $1 \cdot 10^{-8}$  is always excitatory, and augments the tonus, and especially in the rectum increases the number and rigour of contractions in *Loligo pealii*, while the same author observed no effects in the ascidian, *Ciona intestinalis*, as has been mentioned above. MEDWEDEWA (1933) injected 0.1–0.2 ccm. of adrenaline solution (1:1000) into the foot musculature of *Helix*, and observed distinct hyperglycemia; normal sugar content of haemolymph was 0.012%, and this rose to 0.035% half an hour after the adrenaline injection. The same author also reports that the myoneme and ciliary apparatus of *Paramoecium* reacted to the action of adrenaline by the increase of the frequency of contraction, by the rise of the tonus, and eventually by a tetanic contraction. MEDWEDEW (1935) observed intense hyperglycemia in adrenaline-injected *Astacus fluviatilis*, although the variation of effects was great, the blood sugar content of the experimented animals ranging from 85% to 281% of the normal value. Accelerative effect of adrenaline on the oxidative action of isolated cells has been reported by some authors, but denied by others. HODEL (1934) recently observed that adrenaline does not increase the oxidation process of isolated cells in Ringer solution, but an accelerative effect appears when a small amount of serum is added to the medium.

Effect of adrenaline upon metabolism is a complicated problem. It is generally known that a subcutaneous injection of adrenaline invariably produces hyperglycaemia and glycosuria in higher animals, and this occurs even if the animal had been fed on a diet free from carbohydrates; it is then accompanied by increased excretion of nitrogen, indicating that sugar is formed from protein, though other observations do not support this (SCHÄFER 1916). There is also an increase in the metabolic rate as well as in the respiratory quotient, the latter change showing an increased utilization of carbohydrate (BODANSKY 1930, p. 406). GRAY once suggested that a glycoprotein forms the source of ciliary energy in the *Mytilus* gill, while BOYLAND suggested that glycolysis supplies ciliary energy in the *Pecten* gill (GRAY 1928). The effect of adrenaline upon the respiratory quotient of the ciliated epithelium of the oyster gill would therefore be

an interesting subject, and will be determined in a subsequent experiment.

The state of adrenaline in tissues, blood and other body fluids, and the fate of injected adrenaline in the organism are difficult and complicated problems, and little has been known hitherto, but important knowledge has been accumulated in recent years. Concentration of adrenaline in blood has been studied by many investigators, but their results have not been in agreement, and STEWART was skeptic about the various values in literature and maintains that concentration of adrenaline in the arterial blood could not have been greater at most than 1:500,000,000 to 1:1,000,000,000. These concentrations are covered by the range of concentrations of adrenaline examined in our experiments.

Adrenaline in solution is easily oxidized, especially when the solution is alkaline. OLIVER and SCHÄFER had already observed that adrenaline is not destroyed by prolonged contact with blood, nor is it eliminated by the urine. The autoxidation in vitro of adrenaline has often been assumed to be responsible for the inactivation of the drug in organisms. The observations of OLIVER and SCHÄFER, however, suggest the existence of some mechanism for protection of adrenaline from rapid oxidation and consequent inactivation. Knowledge in this line is rapidly increasing, and will clear up many difficulties and discrepancies among authors on the subject. As to the inactivation of adrenaline, the recent writing of BAIN, GAUNT and SUFFOLK may be cited. "— that while adrenaline inactivation proceeds to completion in blood and liver, blood serum and blood plasma, this does not happen in blood alone: in this last an equilibrium is established beyond which no further inactivation of adrenaline can be demonstrated. The equilibrium concentration of adrenaline is proportional to the initial concentration within certain limits. There is also some variation, for a given initial concentration, from sample to sample of blood. Addition of fresh blood to an equilibrium mixture does not result in further inactivation of the remaining adrenaline; whereas addition of more adrenaline to an equilibrium mixture results in some further inactivation, with the establishment of a new equilibrium point. That the equilibria are due, in part at least, to the passage into the blood corpuscles of some of the adrenaline, in which situation it is incapable of exhibiting pressor activity, is suggested by the following facts: firstly, all the adrenaline activity of an equilibrium mixture is present in the plasma; secondly, haemolysed corpuscles from an equilibrium mixture exhibit adrenaline activity, such activity not being shown by control haemolysed corpuscles, or by unhaemolysed corpuscles from an equilibrium mixture; and lastly, addition to fresh plasma of corpuscles

from an equilibrium mixture leads to the appearance of adrenaline activity in the plasma-corpusele mixture." This knowledge is very important in considering the adrenaline action in our experiments, in which the adrenaline was dissolved in sea-water, that has an alkaline reaction which would facilitate inactivation. Adrenaline in sea-water is progressively oxidised, and a red oxidation product is sometimes perceivable. But it may be assumed also that the drug on entering the cells is protected from rapid oxidation and thus retains its activity. Further evidences obtained by recent authors lend support to our assumption: *i. e.* more prolonged maintenance of adrenaline activity in cell interior than would be expected from the alkaline reaction of the solution medium.

In the blood and tissues, the presence of inhibitors of autoxidation has been known and the rôle of different substances such as amino acids, glutathione, ascorbic acid, and guanidine as inhibitors of autoxidation has recently been discussed by BLASCHKO, RICHTER and SCHLOSSMANN (1937).

HEARD and WELCH (1935) report that adrenaline is protected by glutathione, ascorbic acid, and cysteine from autoxidation in biological systems, while the BARRONS and KLEMPERER (1936) report that ascorbic acid in turn is protected from oxidation in biological fluids by the action of glutathione, proteins, and amino-acids, which inhibit copper catalysis. BLASCHKO, RICHTER and SCHLOSSMANN (1937) on the contrary observed the inactivation of adrenaline in rat and guinea pig tissues. The relation of redoxpotentials (MICHAELIS 1929) of the substances under consideration is interesting. The redoxpotential of adrenaline was measured by BALL and CHEN (1933) and  $E_o' = 0.338$  at pH 7 and 30°C. and that of ascorbic acid measured by GREEN (1933) is much lower,  $E_o' = -0.045$  at the same pH and temperature, and as far as a small portion of ascorbic acid remains in reduced form, it will protect the adrenaline from oxidation. The intracellular redoxpotentials of various cells, that have been measured, are lower than that of adrenaline, and would favour the existence of the reductant form of adrenaline-redoxsystem. TERAJ and others studied the effect of redoxsystem on the adrenaline action and their experiments on various organs of cold and warm blooded animals showed the suppression of adrenaline action by quinone and the recovery of its action by the addition of hydroquinone, which they interpret as evidence that adrenaline apparently undergoes a reversible change between an active hydroxy-form and an inactive oxy-form. Sodium bisulfite could be substituted for hydroquinone, and iodine for quinone with the same reversible changes.

In a previous work (1933), I determined the intracellular oxidation-

reduction potential of the ciliated gill epithelium of *Pecten yessoensis*, under anaerobic conditions. As the oxygen tension of the medium in the closed chamber gradually decreased by the oxygen consumption of the enclosed gill tissue, the ciliary movement gradually slowed and at last stopped. The redox-potential at this time was determined by means of vital staining and its decolouration, and from consideration of this value with that of sugars (PREISLER 1927, WURMSER 1930), it was suggested that the energy of ciliary movement might be derived from glycolysis. Adrenaline, which is sympathomimetic and causes hyperglycaemia and glycosuria in higher animals and in some invertebrates, however, inhibits the ciliary activity of the oyster gill as is observed in the present study. Whether adrenaline inhibits glycogenolysis, which is accelerated in higher animals, or whether it inhibits glycolysis remains open to further experimental studies, nervous regulation of ciliary movement here being assumed to be excluded.

#### SUMMARY AND CONCLUSION

1. The effect of adrenaline on the ciliary movement of the gill of the oyster *Ostrea gigas* THUNBERG has been determined.
2. A wide range of concentrations from  $10^{-5}$  to  $10^{-10}$  has been examined.
3. The effect is inhibitory in all concentrations examined.
4. Various problems bearing upon the ciliary movement and the adrenaline effects have been reviewed and discussed.

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## SYMBOLAE ITEOLOGICAE IV

AUCTORE

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(Cum 3 tabulis et 3 figuris in textu)

(Opus acceptum d. XXX m. Aug. a. MCMXXXVII)

- 33) × *Toisochosenia Tatewakii*<sup>1)</sup> KIMURA hyb. nov. (Fig. 1 & Tab. XI).  
= *Chosenia bracteosa* (TURCZANINOW) NAKAI × *Toisusu cardiophylla*  
(TRAUTVETTER & MEYER) KIMURA.

*Arbor* est magna habitu *Toisusu cardiophyllam* simulat trunco circiter 40 cm diametiente (ex TATEWAKI). *Ramuli* annotini nitiduli glaberrimi (juxta nodos autem interdum minutissime puberuli), sub prelo facilius atro-fuscescentes, 10–32 cm longi, superne circiter 2 mm medio 2–3 mm circa basin 4 mm crassi. Internodia in medio ramuli 1.5–2.8 cm longa. *Gemmae* amentiferae ovato-oblongae latere carinatae, superne angustatae atque plus minus complanatae, glaberrimae rufo-brunneae, circiter 11 mm longae 2.5 mm latae; perula gemmae una coriacea ventre libera, marginibus imbricatis, externo glaberrimo interno ciliato. *Cataphylla* sterilium ramulorum membranacea sessilia, prima adaxialia late ovata apice acuta margine integerrima, supra glaberrima subtus praeter basin glabram adpresse villosa, villis marginem superantibus, 9–11 × 6–7 mm magna, secunda abaxialia elliptica circiter 12–13 × 6.0–6.5 mm magna ceterum ut in primis. *Folia adulta* non visa. *Amenta* ♂ (paullum ante anthesin staminibus nondum satis exsertis visa) coetanea oblongo-cylindrica apicem versus angustata, 2.7 cm longa 7 mm crassa, foliato-pedunculata; pedunculi parce pilosi ad 6 mm longi, foliolis 4–6 (–7), quorum primis et secundis caducissimis et forma magnitudine indumentoque iis sterilium ramulorum simillimis, ceteris obovato-oblongis, vere vel anguste oblongis, antice late vel anguste acutis, basi obtusis breviter petiolatis, margine integerrimis, supra fere glabris infra dilute glaucis nunc dense nunc tenuiter adpresse villosis-sericeis vel minute sericeis, 14–20 mm longis 5–8 mm latis, supremis minoribus. *Bracteolae* obovatae vel flabellato-obovatae, apice rotundatae vel truncato-rotundatae integerrimae vel irregulariter undulatae, deorsum interdum late

<sup>1)</sup>Nomen hoc dedi in honorem doctoris M. TATEWAKI florum boreali-japonicae peritissimi qui hanc raritatem detexit et mihi specimina pretiosa largitus est.

cuneatae, intus glaberrimae, extus suprema parte vulgo paucipilosae ceterum glaberrimae, margine ciliatae, 2.0–2.3 mm longae 1.8–2.3 mm latae. *Glandula* una *dorsalis* semper evoluta oblonga 0.3–0.5 mm longa, ventralis omnino nulla. *Stamina* numero varia plerumque 5, raro 3 vel 4, rarissime 6, filamentis liberis et glabris nondum satis exsertis. *Antherae* luteae late ovales extrorsae 0.6–0.8 mm longae 0.7–0.8 mm latae. Grana pollinis normalia nec deformia modo vix variabilia.

Hab. Japonia. Sachalin austr.—Distr. Sikka: ad fl. Mirukunai, (TATEWAKI & TAKAHASHI n. 22489 [typus] 15 Jun. 1936 in Herb. A. KIMURA).

TATEWAKI ill. ad me scripsit hanc in associatione *Choseniae bracteosae* NAKAI et *Toisusu cardiophyllae* KIMURA occurrere in ripa fluminis Miru-

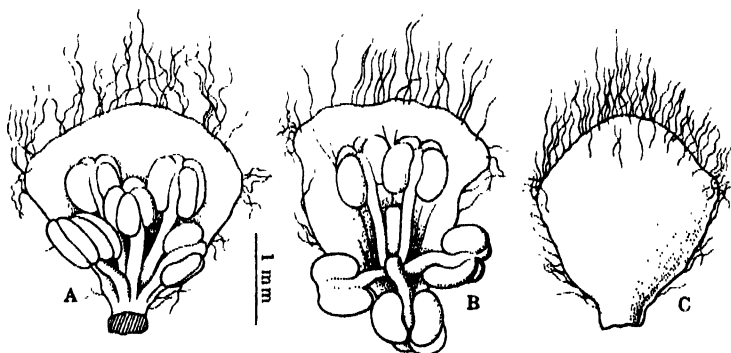


Fig. 1. *Toisochosenia Tatewakii* KIMURA. A Flos ♂. B Idem glandulam dorsalem monstrans. C Bracteola a dorso visa.

kunai; ibi autem et in vicinitate *Toisusu Urbaniana* KIMURA et ejus var. *Schneiderii* KIMURA non adhuc sunt certe detectae, cui rei debet conubium supra propositum. Discrepat a proxime affini *Toisochosenia kamikotica* KIMURA, quam e *Chosenia bracteosa* NAKAI et *Toisusu Urbaniana* KIMURA hybriditate ortam esse opinor, imprimis florum bracteolis obovatis (nec spatulato-oblongis) brevioribus ac latoribus et glandula dorsali semper evoluta.

34) *Salix subreniformis* KIMURA in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 409 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).—NEMOTO, Fl. Jap. Suppl. p. 116 (1936).

Fig. 2.—Descr. ♂: *Fruticulus* habitu ut in ♀, ramulis laevibus glabris brunneis, annotinis 2.5–3 cm longis 4–5 mm crassis, hornotinis foliiferis ad 4.5 cm longis. *Gemmae* steriles ovatae obtusae glaberrimae flavo-brunneae. *Cataphylla* sterilium atque fertile ramulorum late orbicularia apice leviter

retusa, basi cordata, supra marginalibus partibus villosa, subtus undique villosissima, prima  $8 \times 10$ ,  $13 \times 15$ ,  $18 \times 19$  mm etc., secunda  $13 \times 19$ ,  $20 \times 20$ ,  $23 \times 27$  mm etc. magna; petiolis parce villosis in cataphyllis primis 4–10 mm, secundis 8–14 mm longis. *Folia adulta* late vel pure orbicularia apice plerumque retusa nonnumquam rotundata, basi cordata, margine integerrima leviter reflexa, basi solum rarius obscure remote serrata, supra florendi tempore glabra vel antice tantum albo-villosa non stomatifera, subtus dilute glauca, summa parte tantum vel ad costam vel etiam nervos adpresse villosa, superiora  $3.7 \times 4.3$ ,  $4 \times 5$ ,  $4.2 \times 4.3$ ,  $4.5 \times 4.3$ ,  $4.7 \times 5.5$  cm etc, magna, inferiora minora  $0.9 \times 1.1$ ,  $1.4 \times 1.5$ ,  $2.4 \times 2.7$  cm etc. magna; costa supra impressa infra prominente; nervis primariis arcuatis plus minus acrodromis utrinsecus 6–7 sub angulis  $30^\circ$ – $70^\circ$  a costa divergentibus, supra impressis subtus prominulis; secundariis in sicco utrinque paullum elevatis inter primarios transversis crebrisque. *Petoli* supra canaliculati fere glabri vel parce villosi 1.3–2.3 cm longi laminis breviores. *Amenta* ♂ sub anthesi longe cylindrica circiter 6 cm longa 1.5 cm crassa, rhachidibus pubescentibus, ramulos laterales folia circiter 5 (cataphyllis inclusis) ceteris simillima gerentes 2.2–2.6 cm longos inferne glabros superne pilosos terminantia; pedunculis hirsutis 0.9–1.2 cm longis. *Bracteolae* obovatae apice rotundatae utrinque villosae (intus basi glabrae) antice fuscescentes basi in sicco flavo-brunneae, 2.5 mm longae 1.8–2.0 mm latae. *Glandula* una ventralis ovato-oblonga vel oblonga apice obtusissima vel subtruncata 1.0 mm longa 0.5 mm lata. *Stamina* 2, filamentis liberis glaberrimis 5.5–6.0 mm longis; antherae ovae rubrae 0.8–1.0 mm longae. Grana pollinis lutea.

Nom. Jap. *Zinyo-tisimayanagi* KIMURA in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 410 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).

Hab. Japonia. Kuriles.—Ins. Rashuwa: in herbaceous places, Nakadomari, (TATEWAKI & TAKAHASHI n. 14946 [typus ♂] 3 Aug. 1929 in Herb. Fac. Agr. Hokkaido Univ. Imp.).

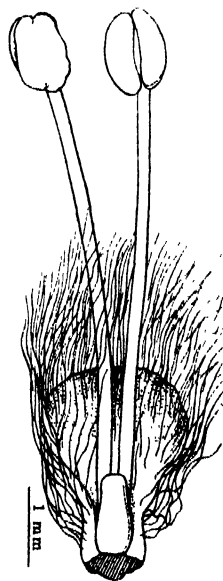


Fig. 2. *Salix subreniformis*  
KIMURA. Flos masculinus.

35) × *Salix Iwahisana* KIMURA hyb. nov. (Fig. 3 & Tab. XII, XIII).  
 = *Salix gracilistyla* MIQUEL × *S. Lackschewitziana* TOEPPFER var. *typica* KIMURA.

*Arbuscula* vel *arbor* ad 8 m alta trunco usque 20 cm diametiente (ex collectore). *Ramuli* teretes graciles, hornotini foliiferi crispo-pubescentes, in partibus juvenilibus molliter albo-villoso-sericeis, in sicco atro-fuscescentes; annotini amentiferi glaberrimi (raro in supremis partibus pubescentibus) in sicco atro-fusci dilute pruinosi, 17–35 cm vel ultra longi, superne 2-mm inferne 4 mm crassi. *Gemmae* amentiferae anguste ellipticae, apice obtusae, ventre minutissime puberulo excepto glaberrimae, circiter 13 mm longae 4 mm crassae; foliiferae anguste ovatae, apice obtusae et complanatae, latere carinatae, dorso vulgo glaberrimae, ventre minutissime pubescentes, in sicco fusco-badiae dilute pruinosa, 4–6 mm longae 2–2.5 mm latae. Cataphylla nondum visa. *Folia recentissima* sub vernatione convoluta utrinque dense adpresseque albo-villosissima, e vernatione relaxata margine infero leviter revoluta quasi modo *S. gracilistylae* MIQUEL. *Folia adulta* chartacea, internodiis 1.0–1.9 cm longis dissita, *inferiora* oblanceolato-oblonga, supra medium latiora, apice breviter acuminato-acuta, basi margine leviter convexo vel rectiusculo acuta, 6–9 cm longa, 2–3.2 cm lata, 2.6–3.0-plo longiora quam latiora, *superiora* oblonga vel lanceolato-oblonga, medio fere latiora, apice acuminata, basi margine convexo obtusissima vel subrotundata, 7.5–11 cm longa, 2.5–3.3 cm lata, 3–3.8-plo longiora quam latiora, margine dentato-serrulata vel denticulata, dentibus glandulosis in medio folii 4–5 pro 1 cm, sursum crebris, supra viridia secus costam solum stomatifera, subtus glauca, utrinque primo pilis longis adpressis acroscopicis rectiusculis cum brevissimis curvulisque mixtis obsita, deinde costa excepta glabrescentia; costa in sicco straminea supra fere plana, primo pilis longis adpressisque cum brevissimis crispulisque mixtis obsita, mox autem longis tantum deciduis, infra vehementer prominente mox glabrescente; nervis primariis tenuibus leviter arcuatis interdum furcatis, utrinsecus 14–16 sub angulis 50°–60° e costa orientibus, supra fere planis infra prominentibus, secundariis tenuissimis in foliis inferioribus irregularibus, in superioribus inter primarios crebre et subparallele transversis quasi modo *S. gracilistylae* MIQUEL, intermediis 0–2. *Petoli* semiteretes supra sulcati tomentosi, subtus primo tomentosi demum glabrescentes, ad 6 mm longi. *Stipulae* bene evolutae, in foliis inferioribus ramulorum oblique vel falcato-lanceolatae, margine serratae, supra virides infra dilute glaucae, utrinque fere glabrae, 5 × 1, 6 × 1.5, 8 × 1.5, 11 × 2.5 mm etc. *magnae*, in superioribus oblique ovatae, apice breviter acuminatae, basi leviter cordatae, margine denti-

culatae, supra virides basi pauci-glandulosae, infra glaucae, utrinque primo pubescentes demum glabrescentes,  $8 \times 5$ ,  $10.5 \times 6.5$ ,  $14 \times 8$  mm etc. magnae.

*Amenta* ♂ praecocia densiflora oblongo-cylindrica, ante anthesin villosissima, aspectu iis *S. Lack-schewitzianae* TOEPFER simillima, sub anthesi 3-4 cm longa 1.8-2.2 cm crassa, basi sessilia bracteofoliis 1-3 anguste ovatis apice obtusis margine integerrimis, utrinque albo-villosis, colore viridibus partim nigrescentibus, circiter 5 mm longis 2-2.5 mm latis suffulta. *Bracteolae* oblongae vel obovato-oblongae, superne acutae, deorsum subcuneatae, utroque margine infero nunc glandulosae nunc fere integrae, dimidia vel maiore parte superiore nigrae, inferiore pallidae, medio interdum rubicundae, utraque facie dense albo-villosae, 3.4-3.6 mm longae 1.3-1.5 mm latae. *Glandula* una ventralis oblonga apice truncata 0.7-0.9 mm longa 0.4 mm lata. *Stamina* 2, filamentis vulgo glabris 8.5-10.0 mm longis, e basi ad  $\frac{1}{2}$ - $\frac{3}{4}$  totae longitudinis vel apicem usque connatis. *Antherae* ovaes, flavae, apice tantum rubicundae, 1 mm longae.

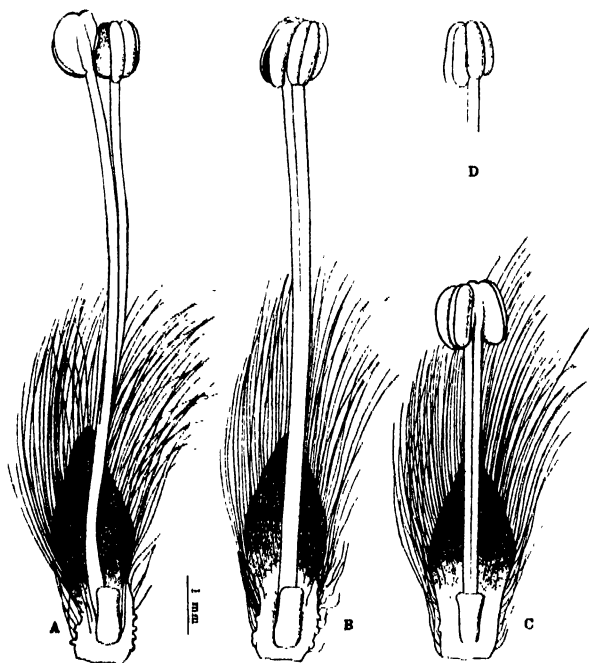


Fig. 3. *Salix Iwahisana* KIMURA. A Flos ♂ ex n. 6641. B Idem ex n. 6642. C Idem ex n. 6641, bracteola fere integra, filamentis nondum satis exsertis. D Anthera a facie.

Nom. Jap. *Yoiti-yanagi* YAMAMOTO nom. nov. in litt.

Hab. Japonia. Ins. Yezo.—Prov. Siribesi: ad ripas fl. Hugoppe, in vicino oppidi Yoiti, (I. YAMAMOTO n. 6641 ♂ fl. [typus] 25 Apr. 1937 in Herb. A. KIMURA, fol. 20 Jul. 1936; n. 6642 ♂ fl. 25 Apr. 1937, fol. 20 Jul. 1936; n. 8049 st. 4 Jul. 1937.—A. KIMURA n. 2753 st. & n. 2754 st. 10 Aug. 1937).



Hanc formam a *Salice gracilistyla* MIQUEL et *S. Lackschewitziana* var. *typica* KIMURA esse hybridam vix ullo dubio caret. Ab illa habet vestimentum ramulorum, folia recentissima utrinque villosissima, adulta supera facie stomatifera, nervos tertios superiorum foliorum crebros et regulares, florum bracteolas antice acutatas nec non antheras figura ovaes. Cum hac communia habet: staturam arborescentem, ramulos saltem hieme pruinosos, sub prelo facilius nigrescentes, foliorum texturam et colorem, stipulas supernas majores et ovatas, amenta villosissima et crassissima, florum bracteolas basi saepe glandulosas, antheras flavas solum apice rubicundas. Character filamentorum figura et magnitudo glandulae inter parentes fere media.—In honorem collectoris IWAHISA YAMAMOTO nominata.

36) *Salix brachypoda* (TRAUTVETTER & MEYER) KOMAROV in Act. Hort. Petrop. XXXIX. p. 49 (1923) (Fl. Austro-Ussur.).—NAZAROV in KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 122, 709 (1936).

Syn. *Salix repens* var. *brachypoda* TRAUTVETTER & MEYER in MIDDENDORFF, Reise Sibir. I. pt. 2, Bot. abt. 2, p. 79 (1856) (Fl. Ochot.).—TRAUTVETTER in MAXIMOWICZ, Prim. Fl. Amur. p. 245 (1859).

*Salix repens* subsp. *rosmarinifolia* var. *flavicans* ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 116 (1867) (Monogr. Salic.).

*Salix repens* var.  $\gamma$  *flavicans* ANDERSSON in DE CANDOLLE, Prodr. XVI. 2, p. 238 (1868).—SEEMEN in ASCHERSON & GRAEBNER, Syn. Mitteleur. Fl. IV. p. 128 (1909).

*Salix repens* FR. SCHMIDT in Mém. Acad. Imp. Sci. St. Pétersb. sér. 7, XII. 2, p. 61 (1868) (Reise Amurl. Sachal.).—KOMAROV in Act. Hort. Petrop. XXII. p. 29 (1903) (Fl. Mansh. II.).—NAKAI in Jour. Coll. Sci. Tokyo Imp. Univ. XXXI. p. 214 (1911) (Fl. Korea. II.).—MATSUMURA, Shokubutsu-Mei-I, ed. 9, II. p. 354 (1916).

*Salix sibirica* NAKAI in Tokyo Bot. Mag. XXXII. p. 30 (1918); Fl. Paiktusan p. 63 (1918); in Bull. Soc. Dendr. France no. 66, p. 10 (1928).—MATSUMURA, Shokubutsu-Mei-I, ed. 10, II. p. 597 (1922).

*Salix sibirica* var. *brachypoda* NAKAI, Fl. Syl. Korea. XVIII. p. 158 (1930).

*Salix finalis* KIMURA in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 451 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).—NEMOTO, Fl. Jap. Suppl. p. 106 (1936). Syn. nov.

Nom. Jap. *Numa-kinuyanagi* NAKAI in Tokyo Bot. Mag. XXXII. p. 30 (1918).—*Sakai-yanagi* KIMURA l. c. p. 452 (1934).

Hab. Japonia. Sachalin austr.—Distr. Sikka: Higasiyama, ad lat. bor.

c. 50° (T. MIYAKE st. [typus *S. finalis*] 28 Aug. 1906 in Herb. Fac. Agr. Hokkaido Imp. Univ.).

*Salicis finalis* specimen originale floribus caret sed certe ad hanc pertinet. Nova civis florum Sachalinensis. *Salix sibirica* KUDO in Jour. Coll. Agr. Hokkaido Imp. Univ. XII. 1, p. 30 est *S. Kudo* KIMURA.

37) *Salix Pet-susu* KIMURA nom. nov.

Syn. *Salix viminalis* (non LINNAEUS) SUGIYAMA, Hokkaido-Zyumokusiryo n. 158 (1890).—MIYABE in Tokyo Bot. Mag. VI. append. p. 8 (1892) (MIYABE & JIMBO, Ainu names of Hokkaido plants p. 8); in Jour. Tokio Geogr. Soc. XIV. p. 54 (1892) (MIYABE & JIMBO, Ainu Names of Hokkaido Plants p. 19).—MATSUMURA, Shokubutsu Mei-I p. 261 (1895); Index Pl. Jap. II. 2, p. 15 (1912).—TUKUBUCHI in Tokyo Bot. Mag. X. p. 124 (1896).—SEEMEN, Salic. Jap. p. 50, t. 9, F—K (1903) quoad pl. ex Jesso et Sachalin.—LÉVEILLÉ in Bull. Acad. Intern. Géogr. Bot. XIV. p. 209 (1904); ibid. XVI. p. 146, 148, 151 (1906).—MIYABE & MIYAKE, Karahuto-Syokubutu-Tyosa-Gaiho p. 17 (1907); Fl. Saghal. p. 428 (1915).—MAKINO & NEMOTO, Cat. Jap. Pl. Herb. Nat. Hist. Dept. Tokyo Imp. Mus. p. 311 (1914).

*Salix viminalis* L. var. *yezoensis* SCHNEIDER in SARGENT, Pl. Wilson. III. p. 158 (1916).—MIYABE & KUDO, Icon. Ess. For. Trees Hokkaido, I. p. 58, t. 17 (1921).—KUDO in Jour. Coll. Agr. Hokkaido Imp. Univ. XII. 1, p. 30 (1923) (Contr. Fl. North. Saghal.); Rep. Veg. North. Saghal. p. 100 (1924).—MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1130 (1925); ed. 2, p. 173 (1931).

*Salix Gmelini* PALLAS var. *yezoensis* KIMURA in litt. ex GÖRZ, Sched. ad fasc. I. Salic. Asiat. p. 16 (1931); in FEDDE, Rep. Sp. Nov. XXXII. p. 387 (1933).

*Salix yezoensis* KIMURA in Tokyo Bot. Mag. XLV. p. 28 (1931); in Sci. Rep. Tōhoku Imp. Univ. 4 ser. Biol. VI. 2, p. 190 (1931) (Contr. Salic. Jap. IV.); in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 430 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).—TATEWAKI, For.-Assoc. & Lign. Fl. Uryu Univ. Exp. For. I. p. 111 (1932).—HARA in Tokyo Bot. Mag. XLVIII. p. 800 (1934).—NEMOTO, Fl. Jap. Suppl. p. 118 (1936).

*Salix serotina* PALLAS var. *yezoensis* KIMURA ex GÖRZ in FEDDE, Rep. Sp. Nov. XXXVI. p. 26 (1934) (GÖRZ, Sched. ad fasc. III. Salicac. Asiat.) in nota ad *S. serotinam*.

Nom. Jap. *Kinuyanagi* (non IWASAKI KWANEN<sup>1)</sup>, Honzôzuhu LXXXI. fol. 17 verso & 18 recto) SUGIYAMA, Hokkaido-Zyumokusiryo n. 158 (1890).--- MIYABE in Tokyo Bot. Mag. VI. append. p. 8 (1892) (MIYABE & JIMBO, Ainu names of Hokkaido plants p. 8).--- *Uraziroyanagi* SUGIYAMA, Hokkaido-Zyumokusiryo n. 158 (1890).--- *Ginyanagi* SHIRAI in Tokyo Bot. Mag. XII. p. 111 (1898).--- *Yezono-kinuyanagi* KIMURA in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 430 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).

Nom. Ainu. *Petsusu* JIMBO in Tokyo Bot. Mag. VI. append. p. 8 (1892) (MIYABE & JIMBO, Ainu names of Hokkaido plants p. 8).--- *Yayaisusu* JIMBO in Tokyo Bot. Mag. VI. append. p. 8 (1892) (MIYABE & JIMBO, Ainu names of Hokkaido plants p. 8).

Huic iterum nomen novum imposui, quod nomen *Salicis yezoensis* cum *S. jessoensis* homonymum esse puto.

var. *angustifolia* (KIMURA) KIMURA nom. nov.

Syn. *Salix yezoensis* KIMURA var. *angustifolia* KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. VI. 2, p. 194 (1931) (Contr. Salic. Jap. IV.); in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 430 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).--- NEMOTO, Fl. Jap. Suppl. p. 118 (1936).

var. *abbreviata* (KIMURA) KIMURA nom. nov.

Syn. *Salix yezoensis* KIMURA var. *abbreviata* KIMURA in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 431 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).--- NEMOTO, Fl. Jap. Suppl. p. 118 (1936).

38) *Salix Kimurana* (MIYABE & TATEWAKI) MIYABE & TATEWAKI in Trans. Sapporo Nat. Hist. Soc. XIV. p. 255 (1936); in Proc. Imp. Acad. Tokyo XIII. p. 26 (1937).--- KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. XII. 1, p. 103 (1937) (Symb. Iteolog. III.).

Syn. *Salix berberifolia* PALLAS var. *Kimurana* MIYABE & TATEWAKI in Trans. Sapporo Nat. Hist. Soc. XIV. p. 84 (1935).

Specimina ampla nuperrime vidi a B. YOSHIMURA et M. HARA loco classico lecta. Floribus variat:

form. *typica* KIMURA nom. nov.

<sup>1)</sup> *Kinuyanagi* ab IWASAKI KWANEN l. c. cum icone bene illustrata et descripta a *Salice Pet-susu* aliena est et speciem certe propriam efficit. Illa salix in Japonia statu culto vulgariter, sed nequaquam spontanee, occurrit, atque ↑ tantum; fieri potest, ut ex Korea antiquis temporibus introducta sit.

Ovaria superne latere dorsali et ventrali parce crispo-pilosa, stipitibus pilosis.

Hab. Japonia. Sachalin austr.— Distr. Sikka: the upper Rukutama, (M. KAWASHIMA [typus] 2 Aug. 1935; n. 1, 16 Jul. 1935).

form. *hebecarpa* KIMURA form. nov.

A typo recedit ovariis dimidia superiore parte plus minusve dense crispo-pubescentibus.

Descr. specim. originalis. — Flores ♀ paullum post anthesin: *Bracteolae* obovatae apice rotundatae utrinque hirsutae circiter 1.8 mm longae 0.9–1.0 mm latae. *Glandula* una ventralis ovata apice truncata 0.6–0.7 mm longa circiter 0.5 mm lata. *Ovaria* 3 mm longa dimidia superiore parte crispo-pubescentia, inferiore glabra; stipitibus pilosis 0.7–0.8 mm longis; stylis 0.5–0.6 mm longis. *Stigmata* bifida, laciniis 0.3 mm longis.

Hab. Japonia. Sachalin austr. — Distr. Sikka: Mt. Sekkaizan, the upper Rukutama, (B. YOSHIMURA & M. HARA [typus form.] 15 Jul. 1937 in Herb. Fac. Agr. Hokkaido Imp. Univ.).

form. *psilocarpa* KIMURA form. nov.

A typo recedit ovariis stipitibusque glaberrimis; ceterum ut in typica.

Descr. specim. originalis. — Flores ♀ paullo post anthesin: *Bracteolae* obovatae apice rotundatae pro maxima parte nigrescentes utrinque hirsutae circiter 1.7 mm longae 1.0 mm latae. *Glandula* una ventralis ovata truncata 0.7 mm longa 0.4 mm lata. *Ovaria* glaberrima 3 mm longa; stipitibus glaberrimis 0.8 mm longis; stylis 0.7 mm longis. *Stigmata* bifida, laciniis circiter 0.3 mm longis.

Hab. Japonia. Sachalin austr.— Distr. Sikka: Mt. Sekkaizan, the upper Rukutama, (B. YOSHIMURA & M. HARA [typus form.] 15 Jul. 1937 in Herb. Fac. Agr. Hokkaido Imp. Univ.).

39) *Salix araeostachya* SCHNEIDER in SARGENT, Pl. Wilson. III. p. 96 (1916).—HANDEL-MAZZETTI, Symb. Sin. VII. p. 60 (1929).—GÖRZ in Bull. Fan Mem. Inst. Biol. (Bot.) VI. 1, p. 1 (1935) (Addit. ad Salicol. Sin.).—SHUN-CHIN LEE, Forest Bot. China p. 189 (1935).

Syn. *Salix tetrasperma* BURKILL in Jour. Linn. Soc. XXVI. p. 533 (1899) (p. p. non ROXBURGH) fide SCHNEIDER.

Gemmae steriles in axillis adutorum foliorum ovatae apice acutae, latere carinatae, fusco-brunneae fere glabrae, circ. 5 mm longae. Perula gemmalis latere ventrali libera et imbricata. Folia adulta supera facie non stomatifera. Petioli eglandulosi.—Propter gemmarum naturam pri-

mitivam est subgen. *Protiteae* KIMURA subjungenda.

Hab. in Sina australi; Yunnan & Szechwan.—Specimina examinata: Yunnan: "Between Muang Hai and Keng Hung, on the banks of the Nam Ha, at 1260 m. altitude", (J. F. ROCK n. 2474 ♀ 15 Feb. 1922); Mengtsze, southeastern mountain forests, 5000', (A. HENRY n. 11250 ♀, tree 10'); Tsing-pian, alt. 1500 m, in ravine, (H. T. TSAI n. 52561 ♀, shrub 14 ft. 9 Jan. 1933); "Shweli River drainage basin to summit of Shweli-Salween water-shed east of Tengyueh, tree 30 ft. on Kuyung plain along watercourses", (J. F. ROCK n. 7697 ♂ Nov. 1922); "Shweli-Salween, 30-40 ft.", (G. FORREST n. 25149 ♂ 1924-25).—Szechwan australis: "Ningyüanfu. Bach bei Sitchi. Baum bis 6 m", (C. K. SCHNEIDER n. 923 ♀ 17 Apr. 1914).—Omnia in Herb. Arb. Arnoldiani conservata.

40) *Salix Cavaleriei* LÉVEILLÉ in Bull. Soc. Bot. France LVI. p. 298 (1909); Fl. Kouy-Tchéou p. 181 (1915); Cat. Pl. Yun-Nan. p. 251 (1917).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 101 (1916).—HANDEL-MAZZETTI, Symb. Sin. VII. p. 61 (1929).—REHDER in Jour. Arnold Arb. X. p. 113 (1929).—SHUN-CHIN LEE, Forest Bot. China p. 192 (1935).

Syn. *Salix polyandra* LÉVEILLÉ in Bull. Soc. Agr. Sci. Sarthe XXXIX. p. 325 (1904) (Bouquet Fl. Chine, 10); in FEDDE, Rep. Sp. Nov. VI. p. 377 (1909); in Bull. Soc. Bot. France LVI. p. 300 (1909).

*Salix Pyi* LÉVEILLÉ in Bull. Soc. Bot. France LVI. p. 300 (1909).

*Salix yunnanensis* LÉVEILLÉ in Bull. Soc. Bot. France LVI. p. 301 (1909).

*Salix tetrasperma* (non ROXBURGH) DIELS in Not. Bot. Gard. Edinburgh VII. p. 91 (1912) (Pl. Chin. Forrest.) sec. SCHNEIDER.

Gemmae nondum satis evolutae visae, perulis ventre liberis et imbricatis. Folia supra facie non stomatifera. Petioli eglandulosi. In subgen. *Protiteam* KIMURA collocanda.

Hab. in Sina australi; Yunnan.—Specimina visa: "nördlich von Yunnanfu zwischen Ssiaomakai und Schinlung bei heisser Quelle bis 4 m", (C. K. SCHNEIDER n. 4030 ♂ fl. 9 Mart. 1914); "Mai. Grand saule buissonnant. Graine noire en épis. Bord de la riviere à Ta-kiao, altit. 2500 m", (E. E. MAIRE n. 521 ♀); loco speciali non indicato, (G. FORREST n. 9609 ♂); "Prope urbem Yünnanfu, ad regionis calide temperatae rivum supra vic. Sandjia copiose. Substr. arenaceo; alt. s. m. ca. 2100 m", (HANDEL-MAZZETTI n. 13062 ♀ & ♂ 5 Apr. 1917).—Omnia in Herb. Arb. Arnoldiani conservata.

41) *Salix azaolana* BLANCO, Fl. Filip. ed. 2, p. 539 (1845); ed. 3, III. p. 188 (1879).=? *Salix tetrasperma* ROXBURGH, Pl. Corom. I. p. 66, t. 97 (1795).

Gemmae glaberrimae, perulis ventre liberis et imbricatis. Folia adulta supra non stomatifera. Petioli eglandulosi. Ad subgen. *Protiteam* KIMURA pertinet.

Hab. Ins. Luzon.—Vidi specimina ♀ & ♂ ex Bosoboso prov. Rizal in Herb. Hort. Bot. Noveboracensis conservata.

#### EXPLICATIO TABULARUM

##### TAB. XI.

*Toisochosenia Tatewakii* KIMURA, Typus.  
Ramuli amentiferi.

##### TAB. XII.

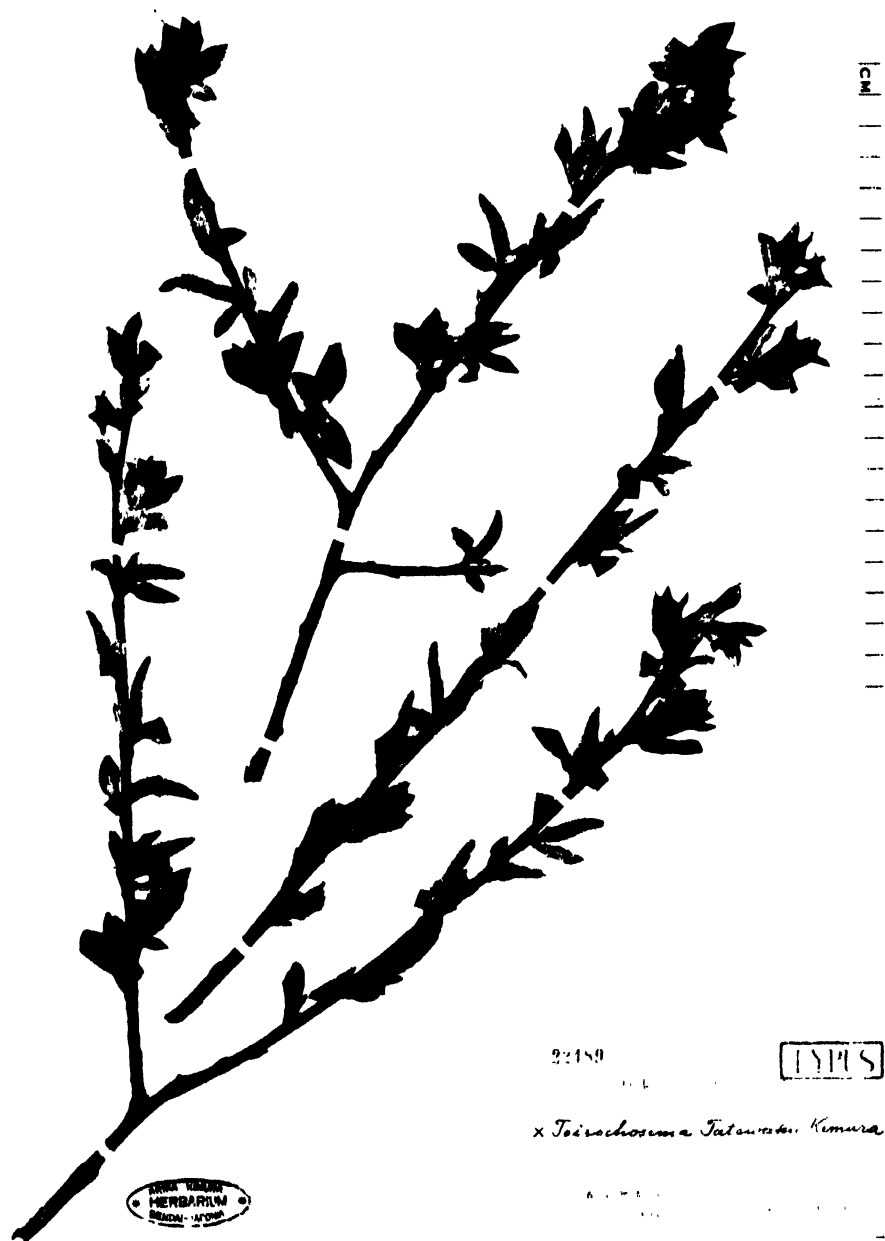
*Salix Iwahisana* KIMURA, Typus.  
Ramuli amentiferi.

##### TAB. XIII.

*Salix Iwahisana* KIMURA.  
Ramuli cum foliis adultis.

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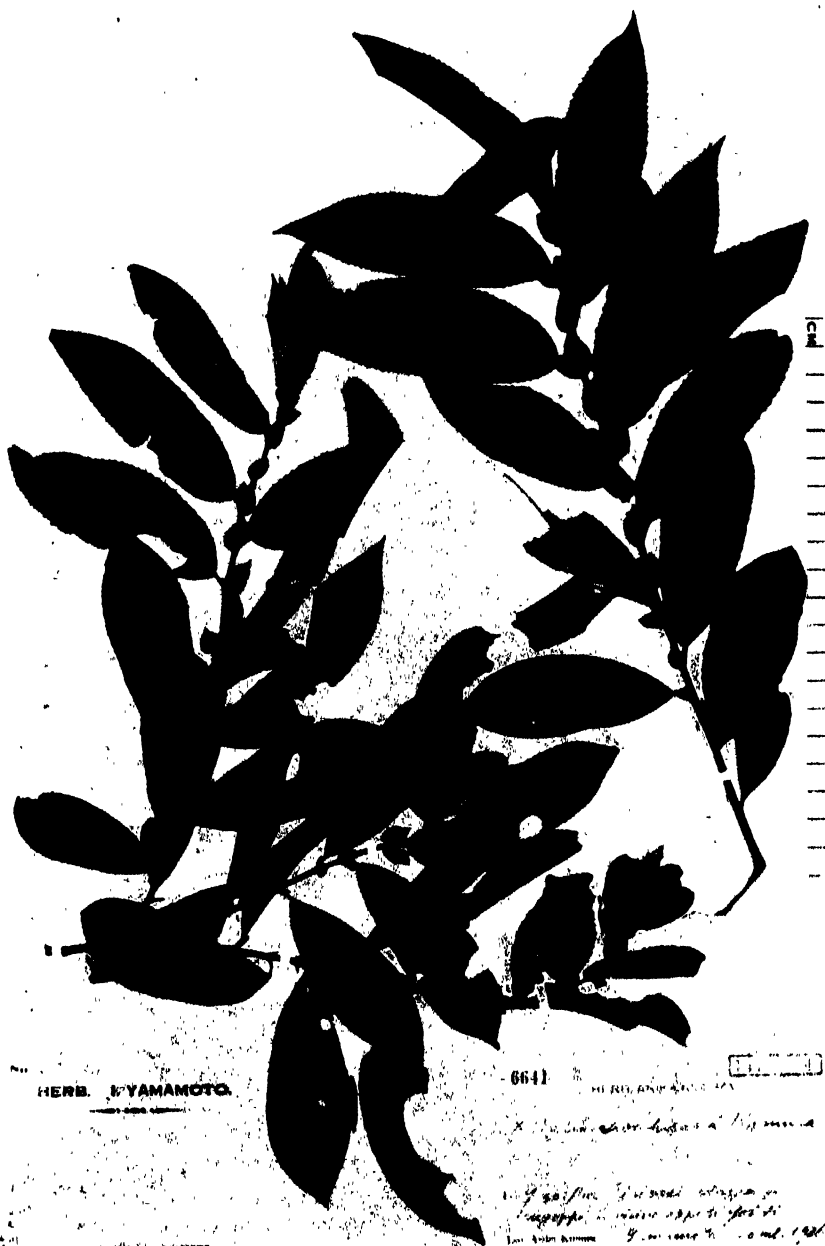














# REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY

## 31. STUDIES ON CHITONS OF MUTSU BAY WITH GENERAL DISCUSSION ON CHITONS OF JAPAN<sup>1)</sup>

By

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(With Plates XIV-XXXIV and seven text-figures)

(Received August 15, 1937)

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### INTRODUCTION

The zoological survey of Mutsu Bay carried out by the staff of the Asamushi Marine Biological Station chiefly during the years 1927-28, has yielded valuable material to increase our knowledge of chitons occurring in that region. The whole collection embraces 21 species as listed below, of which 4 species (6, 7, 13, 19) are new to science and 3 (3, 4, 5)

<sup>1)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 145.

the southernmost species and 2 (18, 21) the northernmost representatives of this group of animals.

1. *Lepidopleurus hakodatensis* THIELE
2. *L. assimilis* THIELE
3. *Tonicella lineata* (WOOD)
4. *T. ruber* (LINNÉ)
5. *T. submarmorea* (MIDDENDORFF)
6. *Spongiopodsia foveolata*, nov. sp.
7. *Mopalia hirsuta*, nov. sp.
8. *Placiphorella stimpsoni* (GOULD)
9. *Acanthochiton rubrolineatus* (LISCHKE)
10. *A. achates* (GOULD)
11. *Cryptoplax japonica* PILSBRY
12. *Ischnochiton comptus* (GOULD)
13. *I. paululus*, nov. sp.
14. *Ischnopodsia hakodadensis* ('CARPENTER' PILSBRY)
15. *Lepidozona coreanica* (REEVE)
16. *L. albrechti* (SCHRENCK)
17. *L. mertensi* (MIDDENDORFF)
18. *Rhyssoplax kurodai* (IS. TAKI et IW. TAKI)
19. *R. tectiformis*, nov. sp.
20. *Liolophura japonica* (LISCHKE)
21. *Lucilina amanda* THIELE

According to THIELE's system the class Loricata is composed of 6 families and 43 genera, and the species listed above belong to 6 families and 11 genera, that is to say, they are distributed among all families of this class.

Of all the species collected from Mutsu Bay, 14 species (1, 3-5, 8-12, 14-16, 18, 20) are those found on the Pacific coast, 16 (1-6, 8-12, 14-16, 18, 20, 21) on the coast of the Japan Sea and 4 (6, 7, 13, 19) are peculiar to that locality. This means that the predominating species are common ones occurring along the coast of the main island of Japan and 2 species (2, 21) are those strictly confined to the Japan Sea and 1 (17) is that uncertain about its distribution in other regions of Japan. Therefore it is hard to say which faunistic region Mutsu Bay belongs to, because it does not show any feature of characteristic of its own. As the warm Tugaru current flows through the straits from the Japan Sea to the Pacific, the fauna of Mutsu Bay seems to be much influenced by that current. Thus *Lepidopleurus assimilis* (2) and *Lucilina amanda* (21) occur

in Mutsu Bay as well as in the Japan Sea. Furthermore in Mutsu Bay the number of species found in the Japan Sea exceeds that found in the Pacific coast by two species. It seems more reasonable to consider Mutsu Bay from the chiton fauna as a region of the Japan Sea than as that of the Pacific.

The chiton fauna of Mutsu Bay are composed of forms of the circumpolar, the northern Pacific, the temperate zone and the tropical regions. Of these elements *Tonicella ruber* (4) may be mentioned as the circumpolar species; the 2 species of *Lepidopleurus* (1, 2), the 2 species of *Tonicella* (3, 5), *Placiphorella stimpsoni* (8), *Ischnoradsia hakodadensis* (14) and the 2 species of *Lepidozonia* (16, 17) may be regarded as peculiar to the northern Pacific; the 2 species of *Acanthochiton* (9, 10), *Cryptoplax japonica* (11), *Lepidozonia coreanica* (15), *Rhyssoplax kurodai* (18), *Liolophura japonica* (20) and *Lucilina amanda* (21) are the temperate zone forms; *Ischnochiton comptus* (12) is a member of the tropical species. The remainders are endemic to this locality.

The genera *Lepidopleurus*, *Ischnochiton* and *Acanthochiton* are world-wide in their distribution, although the first is usually found in the deep seas, and the latter two are known as the representatives of the littoral forms. *Tonicella*, *Spongioradsia*, *Mopalia* and *Placiphorella* are only known from the circumpolar region; *Cryptoplax* appears to flourish better in the coral reef of tropical Australia; *Ischnoradsia* and *Liolophura* show the bipolarity in their distribution occurring in the temperate zones of the both hemispheres, and *Lucilina* is one of the representatives of the Indo-west-Pacific region and Mutsu Bay may be regarded as the northern boundary of its distribution.

The species of chitons described in the following pages were collected by the members of the Asamushi Marine Biological Station, supplemented by the specimens from other sources including those collected by my brother, IWAO TAKI.

I am greatly indebted to Prof. S. HÔZAWA, who has given me a rare privilege to work out the valuable material and to those gentlemen, Dr. K. KOKUBO, Dr. S. TAKATUKI, Mr. H. SATÔ, Mr. KAMADA and Mr. ITÔ, who collected the materials at Mutsu Bay. I must also express my hearty thanks to my friends too many to mention here, especially to Messrs. EDWIN ASHEY, KYÔZI AWAYA, KIKUTARÔ BABA, J. R. M. BERGENHAYN, DAVY J. DEAN, KEN'ITI EBINA, SIGEO EMURA, YOSINE HADA, KAZUITSU HATAKEDA, SINTARO HIRASE, HUZIO HIRO, YOSIYASU HOSUYAMADA, MASARU HORI, ATUSI HUZITA, TADASI HUZITA, TAKEO IMAI, KEN'ITI INA-



ZAWA, TAKASI INO, TUNEITI KAMITA (and his pupils), TOSIHARU KAMOHARA, KÔITIRO KANDA, SUEO KANEKO, TADAO KANO, KANZAEMON KIKUTI, TORAITIRÔ KINOSITA, SIGEO KOBASI, HISAMATU KOHORI, TOKUBEI KURODA, DANIEL B. LANGFORD, JOHN S. MACKAY, MOITIRÔ MAKI, DENZABURÔ MIYAZI, TAKUÔ MIMURA, TAMEZÔ MORI, ZYUNITI MORITA, YAITIRÔ OKADA, KIYOSI OKAMOTO, SIRÔ OKUDA, KATURA ÔYAMA, TAKAHIDE SASAMORI, KAORU SASAMOTO, SYUHEI SONEHARA (and his pupils), RYÛSYÔ SUGIMOTO, D. THAANUM, KEIZÔ TAKAHASI, SADAÊ TAKAHASI, KITARU TAKAGI, SIGERU TAKAGI, KIYOSI TAKEWAKI, RYÛZÔ TANAKA, GENZÔ TOBA, TOMOE URITA, RENZI WADA, WASABURÔ YAGURA, TOMOSABURÔ YAMADA, HIROSI YOSIDA and Miss YAEKO YAMAMURA, whose incessant contributions to the collection of material have enabled me to get a general aperçu of the fauna of the western Pacific chitons. Besides the collection at the Museum of the Zoological Institute, Science Faculty, Tokyo Imperial University, other material came from the "Sôyô Maru" expedition, from the Oceanographical Institute, Tohoku Imperial University, Onagawa, from the Saitô Hôonkai Museum and from the Tokyo Science Museum.

Throughout the course of the present investigation suggestions were received from Drs. N. YATSU and S. TANAKA, to whom I gratefully acknowledge my indebtedness.

#### DESCRIPTION OF SPECIES

##### Class LORICATA

##### Order I LEPIDOPLEURIDA

##### Family LEPIDOPLEURIDAE

##### Genus LEPIDOPLEURUS Risso 1826

##### 1. *Lepidopleurus hakodatensis* THIELE 1909

Pl. XIV, fig. 1; Pl. XVI, figs. 1-4, 6-8, 14; Pl. XVII, figs. 6-8.

##### *Lepidopleurus hakodatensis*

THIELE (1909) Zoologica, Heft 56, S. 10-11, Taf. 1, Fig. 11-20.

Body small, elongate elliptical in outline, strongly vaulted at the back; valves of nearly equal breadth; girdle moderately wide and widest at the middle part of the body.

Head valve semicircular in outline, fairly elevated at the apex, ornamented with fine, flattened, round granules, tending to give a form of distinct radial riblets, especially emphasized at the periphery.

Median valves nearly equal in breadth, oblong in shape, exceedingly

wider than long, thin, fragile, not beaked, strongly arched at the back; lateral area not raised, but distinctly demarcated from the central area by the same sculpture as the head valve; central area with fine granular, longitudinal lirae, their anterior edges protrude a little, though never form distinct denticles; sutural laminae small, hyaline, triangular in shape; sinus wide, flat; interior of valves almost smooth; eaves narrow, a little porous in the tegmental portion.

Tail valve semicircular in outline, almost equal to the head valve in width; mucro nearly central, conspicuous, projecting posteriorly but neither raised nor pointed; central area fairly vaulted with sculpture similar to that of the median valves; posterior slope slightly concave, sculptured like the head valve, margins of tegmentum finely crenulate throughout.

Girdle not too much narrow, widest at about the middle of the body; perinotum scales exceedingly minute, flat, oval in shape, narrowing toward the tip, rounded at the base, with 4 to 6 feeble lines at the apical portion, measuring 72 to 84  $\mu$  in length, 45 to 46  $\mu$  in breadth; among these scales long, straight, more often slightly curved, smooth, pointed spines are interspersed, being 120 to 130  $\mu$  long, 16 to 24  $\mu$  thick (pl. XVI, fig. 6). Hyponotum narrow, with a covering of excessively minute, roughly pentagonal scales, often marked with 2 or 3 quite feeble striae near the apical portion, measuring 18 to 52  $\mu$  in length, 26 to 30  $\mu$  in breadth, showing a tendency to grow long toward the margin so as to form elongate, pointed, more distinctly striated spicules, measuring 100–120  $\mu$  in length, 20–30  $\mu$  in breadth; marginal spines small, straight, smooth, acute at the tip.

Radula small; central tooth broad, truncated at the front end with a well developed cusp, constricted a little near the base, slightly sinuated at the posterior end; centrolateral thin, divided into two lobes by a longitudinal ridge at about the middle, outer lobe shallowly notched at the anterior angle, smooth, sharp in front; inner lobe often extending anteriorly so as to form a small cusp; basal plate oblong; major lateral bicuspidate, outer one much thicker, longer than inner one; stalk thick, twisted strongly at about the middle; inner small-lateral squarish in outline; major uncinus long oar shape, dilated and cusped at the anterior edge; outer marginal oblong, much longer than wide.

All valves light yellow in colour, freckled here and there with easily removable coating of dark brown, although disarticulated valves nearly white; interior of valves also whitish; girdle brownish yellow.

Ctenidia, merobranchial adanal; gills 13 or 14 on one side, ranging

from the 6th to the tail valve.

Remarks: As pointed out by THIELE, the present species bears a close resemblance to *L. assimilis* in all features, the distinctive differences of these two species being found in the shape and the arrangement of the tegmental granulations, in the structure of radula. THIELE figured the radula of this species and stated that the wing-like process of the centro-lateral extends anteriorly so as to embrace the basal part of the major lateral, whereas such a broad expansion can not be detected in the material before me. What he observed may be some structure other than the radula.

Size: Head valve 3.5 mm; 4th valve 4 mm; tail valve 3.5 mm in breadth. Body 9 mm in length, estimated from the curled specimen.

Locality: 1 specimen, off Imabetu, Station 113, no. 2379, collected by S. HÔZAWA in July, 1929.

Distribution: Gulf of Amur; Hakodate (type locality, THIELE); Mutsu Bay; off Yokohama.

## 2. *Lepidopleurus assimilis* THIELE, 1909

Pl. XIV, fig. 2; Pl. XVI, figs. 5, 9-13, 15; Pl. XVII, figs. 9-11.

### ? *Chiton (Leptochiton) concinnus*

GOULD (1859) Proceedings of the Boston Society of Natural History, Vol. 7, p. 164 (not *Chiton concinnus* of SOWERBY, 1840).

— (1860) Otia Conchologica, p. 117.

### ? *Leptochiton concinnus*

CARPENTER, MS. p. 3, (fide PILSBRY)

SCHRENCK (1867) Reisen und Forschungen im Amur-lande, Bd. 2, S. 599.

DALL (1878) Proceedings of the United States National Museum, pp. 316, 318.

— (1878) Bulletin United States National Museum, Vol. 1, p. 100.

DUNKER (1882) Index Molluscorum Maris Japonici, p. 158.

### ? *Lepidopleurus concinnus*

PILSBRY (1892) Manual of Conchology, Vol. 14, p. 11.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 113.

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 11-12.

### *Lepidopleurus assimilis*

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 11-12, Taf. 1, Fig. 30-39.

Body small, oblong, nearly equal in width throughout the entire length; shell elevated, regularly arched, though not carinated; girdle very narrow, nearly equal in breadth in all parts; eaves comparatively broad, smooth, its tegmental portion brownish in colour.

Head valve crescentic in outline, rather flat at the apex with numerous, closely placed, radial series of minute, flattened, round granules, which

arranged rather irregularly near the apex, though forming distinct ribs at the periphery.

Median valves nearly oblong in shape, exceedingly wider than long, neither beaked nor carinated; jugum not defined; granules of central area nearly round, flat, distinctly separated from one another, arranged transversely as well as longitudinally, forming slight denticulations at the front edge; lateral area not elevated, though distinctly defined, sculptured like the head valve, while granules less regularly arranged at the dorsal region than those of the head valve, showing a tendency to form distinct radial riblets at the periphery; sutural laminae thin, small, triangular in outline, with a shallow sinus between them. Interior of valves almost smooth; lateral region shallowly dented, marked with feeble concentric lines of growth; pleural region covered with thick, smooth hypostracum, that makes it distinct from other adjacent regions; jugal region striated closely with short, fine, transverse lines except for the front region.

Tail valve semicircular in outline, somewhat broader than the head valve; central area sculptured like the median valves; posterior area slightly concave; mucro situated near the centre with an obtuse end; eaves rather broader than that of any other valve; interior of valve smooth, with a thick layer of hypostracum; there are 4 muscle impressions at the mucronal region.

Girdle very narrow, almost equal in width in all parts, covered with minute scales, which are generally oval shaped, bluntly pointed at the tip, slightly curved ventrally, marked distally with 10 to 14 strong striations on the dorsal surface, being 80 to 86  $\mu$  in length, 50 to 55  $\mu$  in breadth; marginal spine long, smooth, nearly straight or slightly curved, interspersed among the marginal spicules, measuring 156 to 165  $\mu$  in length, 21 to 23  $\mu$  in thickness; hyponotum scale elongate oval in outline, distinctly striated with 5 to 8 longitudinal lines, measuring 60 to 90  $\mu$  long, 30 to 40  $\mu$  broad, growing longer toward the margin passing over to an oblong marginal spicule, which has a length of 190  $\mu$  and a breadth of 33  $\mu$ , striated like the hyponotum scales.

Radula very small; central tooth of nearly oblong shape, narrowing anteriorly with obtusely pointed cusp at the tip, weakly bilobed at the posterior end; centro-lateral somewhat rolled inward on both edges, produced and cusped at the anterior edge, slightly notched on both sides of cusp, with remarkably small basal-plate at about the middle; major lateral much small, delicate, arranged closely with one another, stalk twisted at about the middle, having a broad extension at the base, cusp with a small

process on the outside near the pointed end; inner small-lateral squarish; major uncinus of long oar shape with a small basal plate; inner marginal oblong in shape, having a triangular extension at the middle part so as to cover the basal part of the major uncinus; middle marginal squarish in outline, bilobed a little at the posterior end, articulating with the anterior edge of the tooth situated immediately behind; outer marginal trapezoid in shape, much wider than long, becoming narrower distally.

All valves with pale orange-yellow or ochraceous-buff, usually fleckled here and there with a dark coating; girdle uniformly coloured with cream-buff.

Ctenidia, merobranchial, without a space between the last ctenidium and the anus, 9 (body length 9.5 mm) to 12 (body length 10 mm) on one side, reaching the tail from the 6th valves.

Remarks: THIELE described *L. hakodatensis* from a specimen collected by HILGENDORF at Hakodate and others from Yokohama and the Gulf of Amur. He then was of the opinion that the characteristic features of *L. concinnus*, reported by GOULD from Hakodate, do not agree well with those of *L. hakodatensis* and furthermore the name "*concinnus*" was applied already by SOWERBY to another species, so that even if this species be identical with GOULD's species, the new name must be proposed.

It is very difficult to determine decidedly GOULD's species from his rather insufficient description only. However careful examination of his descriptions seems to reveal that this form should refer to *L. assimilis*; for these two forms so well agree with each other in most features of shells, in the girdle and also in body size.

While entirely agreeing the descriptions and figures of *L. assimilis* given by THIELE with the specimens from Mutsu Bay except for the details of the radula, in which, contrary to his statements, the central tooth is oblong and becomes a little narrower anteriorly, truncated at the base; the centro-lateral is produced and distinctly cusped at the tip and never develops a thin plate in front so as to embrace the basal part of the major lateral. These differences concerning the structure of radula will be attributable to certain extent to the difficulty of observation, due to the fineness of the radula.

THIELE took a small accessory process on the inner side of the cusp in the major lateral for a residue of the connecting thread with the opposite side of the cusp in the radular sheath. However it may be more reasonable to consider it as a vestige of the inner cusp, which is often developed well in several other species of the genus.

*Size*

BODY		VALVES		
LENGTH	BREADTH	HEAD	TAIL	4TH
6.0 mm	2.7 mm	2.7 mm	2.8 mm	3.35 mm
9.5	5.0	2.6	2.7	3.25
10.0	6.0	1.6	1.7	2.00
		2.25	2.25	2.50
		2.10	2.25	2.50

Locality: 1 specimen off Kamome-sima, 2 specimens off Kanida collected by S. TAKATUKI in July, 1927; 6 specimens collected by IWAOTAKI on August 11, 12 and 15, 1930, at the depth of about 30 m off Yunosima.

Distribution: Near Saghalien (P. SCHMIDT); near Vladivostok (P. SCHMIDT); Hakodate? (STIMPSON); Mutsu Bay.

## Order II CHITONIDA

## Family 1 LEPIDochITONIDAE

## Subfamily A LEPIDochITONINAE

## Genus TONICELLA CARPENTER 1873

3. *Tonicella lineata* (WOOD, 1815)

Pl. XIV, fig. 12; Pl. XVIII, figs. 9 15; Pl. XIX, fig. 2.

*Chiton lineatus*

WOOD (1815) General Conchology, or a description of shells arranged according to the Linnean system, p. 15, pl. 2, figs. 4, 5.

SOWERBY (1847) Conchological Illustrations, fig. 77.

REEVE (1847) Conchologia Iconica, Monograph of the genus Chiton, Vol. 4, pl. 7, fig. 33, species 33.

CARPENTER (1857) Report of the British Association for the Advancement of Science, 1856, pp. 208, 214, 223.

— (1864) Ibidem, 1863, pp. 523, 648, 684.

*Chiton (Stenosemus) lineatus*

MIDDENDORFF (1847) Mémoires l'Académie Impériale des Sciences de Saint-Petersbourg, Ser. 6, Tome 6, pp. 77, 82, 84, 86, 90, 91, 173, Taf. 12, Fig. 8, 9, (Malacologia Rossica, pp. 109-112).

*Tonicia lineata*

CARPENTER (1857) Rep. Brit. Assoc. Adv. Sci., 1856, p. 317.

CARPENTER (1864) Ibidem, 1863, pp. 648, 684.

ADAMS, H. and ADAMS, A. (1858) The genera of recent Mollusca, Vol. 1, p. 474.

*Lepidochitona (Tonicella) lineata*

DALL (1921) Smithsonian Institution United States National Museum, Bulletin 112, p. 188.

*Tonicella lineata*

CARPENTER, MS., p. 38, (fide PILSBRY).

DALL (1878) Proceedings of the United States National Museum, Vol. 1, pp. 296, 326.

— (1878) Smithsonian Institution United States National Museum, Bulletin 1, pp. 78, 108-109, pl. 1, fig. 5.

WOOD and RAYMOND (1891) Nautilus, Vol. 5, p. 58.

PILSBRY (1892) Manual of Conchology, Vol. 14, pp. 42-43, pl. 11, figs. 25-28.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 114.

WISSEL (1904) Zoologische Jahrbücher, Abt. Syst., Bd. 20, S. 592-594, Taf. 22, Fig. 1-4; Bare Island.

BERRY (1917) Proceedings of the Californian Academy of Sciences, Ser. 4, Vol. 7, No. 10, p. 234.

— (1922) Ibidem, Vol. 11, No. 18, pp. 433-435, pl. 2, figs. 1-5.

PACKARD (1918) University of California Publications in Zoology, Vol. 14, No. 2, p. 292, pl. 34, fig. 3.

CHACE, E. P. and CHACE, E. M. (1919) Loricaria, Vol. 2, No. 6, p. 43.

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 4, Nr. 87.

*Tonicella lineata*

THIELE (1893) Das Gebiss der Schnecken, Bd. 2, Lief. 8, S. 390, Taf. 32, Fig. 3.

*Tonicella submarmorea*

THIELE (1893) Ibidem, S. 389, Taf. 32, Fig. 2.

Body of moderate size, oval in shape, broadest at about the posterior third of the entire length; shells fairly elevated, more or less carinated at the back; tegmental surface smooth, characteristically marked with dark-brown lines bordered above with white; girdle rather narrow, leathery.

Head valve of crescentic shape, smooth except for growth lines, solid, thick, straight at the posterior edge, with low but distinct apex, marked concentrically with somewhat irregular, rather zigzag or wavy, white lines upon the ground colour of light reddish; slits range from 8 to 10 in number; slit-rays radiated from the apex to the slits in correspondence with the number of the slits, provided with a series of small pores.

Median valves roundly arched or subcarinated, beaked, oblong in shape with smooth surface, having similarly coloured longitudinal lines; lateral area scarcely raised, indistinct, sloping obliquely backward; jugal area not defined in structure, though often marked with a narrow triangular, whitish line, or bordered with pink or reddish-brown; sutural laminae short, broad, white, thick, crescentic in shape, separated by a deep, narrow, angular sinus; eaves small, spongy; teeth short, but sharply cut with a shallow

slit on each side; slit-rays provided sparsely with small round pores, elongated and crowded near the slit; callus slightly elevated at the border between the central and the lateral areas; the greater part of the interior of valves nearly smooth except for the jugal area, which are striped with numerous faint, transverse, short lines.

Tail valve oval shaped, always smaller than the head valve, its tegmental margin with an indication of false beaking in front; mucro not elevated, blunt, much anteriorly situated; posterior area steep and nearly straight; sutural laminae oblong in shape with a shallow, rather wide sinus between them, having slits, 8 to 10 in number, coloured like the head valve.

Interior of valves tinged with rose colour at about the middle part and with white near the peripheral areas.

Girdle rather narrow, leathery with the ground colour of light brownish yellow, tessellated indistinctly with brownish; perinotum loosely beset with small, short, smooth, variously shaped, spinules, containing sometimes minute granules, having a length of 40 to 60  $\mu$ , a breadth of 12 to 20  $\mu$ , intermingling with minute, nearly straight, hyaline spicules, which are less in number than spinules, measuring 35  $\mu$  in length, 5  $\mu$  in thickness; hyponotum covered by thick, short, flat spinules, rather larger than that of the perinotum, obtusely pointed at the tip, faintly fluted distally with 4 to 5 striae, 70  $\mu$  long, 30  $\mu$  broad.

Radula: Central tooth elongate-oblong in outline, dilated at the anterior half with round end, faintly sinuated at the middle on the anterior edge, truncated at the posterior end; centro-lateral ridged axially at about the middle, bialate at the outer edge, basal plate thick, oblong in shape, obliquely placed and distinctly protruded outwardly, anterior edge smooth, sharp, not cusped, but a small extension at the middle, inner edge nearly straight; major lateral strongly developed, stalk thick, provided with a small wing at the anterior end on either side and two shallow axial grooves on the ventral side, base slightly twisted, truncated at the end; innermost of three cusps separated from middle one by a broad notch, while outer and middle ones divided by a shallow notch, outer one the thickest, to which middle one nearly equal in length or somewhat longer than it; inner small-lateral broad, roughly triangular in shape; peculiarities of major uncinus found in the remarkably long, triangular basal plate and considerably numerous fine cusps at the cutting edge, 50 or more in number, closely arranged along the edge; outer-marginal broad with a slight sinuation at the anterior edge, posterior edge protruding a little backward; middle-marginal oblong shaped, articulating closely with triangular inner-marginal.



Ctenidia, with a wide space between the last ctenidium and the anus, occupying almost entire length of the foot, but slightly separated from both ends of the foot, 16 on one side in the specimen 10 mm long, 25 or 26 in the specimen 30 mm long.

Remarks: The present species is one of the handsomest chitons found in the Pacific region. It has drawn much attention of many authors and was fully described by MIDDENDORFF (1847) and PILSBRY (1892) with respect to the characteristic features of the shell. DALL (1878) and THIELE (1893) figured respectively the radula of this species and WISSEL (1904) revised the results of the latter author, though all the figures and the descriptions are far from being satisfactory, THIELE's results agree better with the preparations before me than WISSEL's. The discrepancies of these results of the previous authors seem to be caused by the different angles from which the material is seen. The structure of this organ is shown in detail in Pl. XIX, fig. 2.

The animal usually grows over 35 mm in length, though the specimens under my examination are very small. Such individuals are never found in the littoral zone but are always collected at some depths.

It is interesting to note that the flatter the specimens, the farther south do they occur and that the specimens from Mutsu Bay agree well with forms from Alaska and northern Canada regarding the divergency of the shell.

### *Size and divergency*

BODY		SHELL				AUTHORS
LENGTH	BREADTH	HEAD	6TH	TAIL	DIVERGENCE	
37.0mm	20.0mm	—	—	—	120°	PILSBRY
30.5	—	—	—	—	—	PACKARD
30.0	15.0	—	—	—	—	PILSBRY
30.0	—	—	—	—	120°	MIDDENDORFF
16.0	10.5	6.00mm	8.80mm	5.00mm	120°	TAKI (Mutsu Bay)
—	—	3.75	4.80	2.80	—	
—	—	3.30	4.40	2.75	100°	
5.4	—	2.50	3.10	1.90	—	

Locality: Off Kanida, 3 specimens (no. 1716) collected by S. TAKATUKI in July, 1927; 1 specimen at Yunosima by IWAOKI on August 15, 1930.

Distribution: Bering Straits south, on both coasts; from Japan and the Okhotsk Sea to the Bay of Monterey, California including whole the Aleutian Islands.

Bering Straits;

Norton Sound (BERRY), Aleutian Islands (PILSBRY).

Asiatic coast;

Siberia; Plover Bay (BERRY), Okhotsk Sea (DALL, PILSBRY).

Japan;

Hokkaidô, Okusiri-zima (T. KINOSITA), Honsyû, Mutsu Bay.

West coast of North America;

Alaska (DALL), Sitka; Unalaska (MIDDENDORFF), Forrester Island (BERRY).

Canada;

Vancouver (collection in the Museum, Tokyo Imp. Univ. Zool. Inst.).

U. S. A.;

Washington, D. C., California, Bonita Point, San Francisco Bay (WOOD and RAYMOND, PACKARD), Monterey Bay (PILSBRY, collection in the Museum, Tokyo Imp. Univ. Zool. Inst.), San Miguel Island, Santa Barbara (BERRY), San Diego (DALL), San Pedro (Pleistocene fossil, CHACE; BERRY).

#### 4. *Tonicella ruber* (LINNÉ, 1767)

Pl. XIV, fig. 3; Pl. XVII, figs. 1-4; Pl. XVIII, figs. 1-5.

##### *Chiton ruber*

LINNÉ (1767) *Systema Naturae*, 12th ed. p. 1107.

LOWE (1825) *Zoological Journal*, Vol. 2, p. 101, pl. 5, fig. 2.

SOWERBY (1839) *Conchological Illustration*, Chiton, figs. 103, 103 a, 104.

GOULD (1841) *Report on the Invertebrata of Massachusetts*, p. 149, fig. 24.

REEVE (1847) *Conchologia Iconica*, Monograph of the genus *Chiton*, Vol. 4, pl. 26, fig. 175.

FORBES and HANLEY (1853) *A List of British Mollusca and Their Shells*, Vol. 2, p. 399, Pl. 59, fig. 6; pl. AA, fig. 6.

HANLEY (1855) *The Shells of Linnaeus, Ipsa Linnaei Conchyliæ*, p. 17.

JEFFREYS (1865) *British Conchology*, Vol. 3, p. 224.

— (1869) *Ibidem*, Vol. 5, p. 199, pl. 56, fig. 4.

BINNEY (1870) *GOULD's Report on the Invertebrata of Massachusetts*, 2nd ed., p. 260, fig. 523.

*Chiton (Stenosemus) ruber*

MÜLLENDORFF (1848) Mémoires de l'Académie Impériale des Sciences de Saint-Petersbourg, Ser. 6, Tome 6, pp. 81, 83, 87, 88, 90, 92, 181-184, Taf. 12, Fig. 5; (Malacozologia Rossica, pp. 117-120): Eismeer Küste des Russischen Lapplandes: Grönlandische Küste: Norwegische Küste.

*Leptochiton ruber*

ADAMS, H. and ADAMS, A. (1858) The Genera of Recent Mollusca, Vol. 1, p. 473.

*Chiton (Lepidopleurus) ruber*

JEFFREYS (1865) British Conchology, Vol. 3, p. 210.

*Boreochiton ruber*

SARS, G. O. (1878) Mollusca Regionis Arcticae Norvegicae, p. 116, Tab. 8, figs. 4 a-1; Tab. 11, figs. 3 a-c; Northern seas, whole coast of Norway, low water to 40 fms.

*Trachydermon ruber*

CARPENTER (1873) Bulletin of the Essex Institute, Vol. 5, p. 153.

DALL (1878) United States National Museum, Bulletin 1, pp. 102-104; Kamchatka; in Alaska from the Pribiloff Islands to Attu and southward to Sitka; Bering Straits northward.

— (1878) Proceedings of the United States National Museum, Vol. 1, p. 320.

PILSBRY (1893) Manual of Conchology, Vol. 15, p. 65, pl. 15, fig. 25.

*Ischnochiton (Trachydermon) ruber*

PILSBRY (1892) Manual of Conchology, Vol. 14, p. 80, pl. 7, figs. 50-56.

*Lepidochitona (Tonicella) ruber*

DALL (1921) United States National Museum, Bulletin 112, p. 188. Arctic Ocean to Monterey, California.

*Lepidochiton (Tonicella) ruber ruber*

JOHNSON, C. W. (1934) Proceedings of the Boston Society of Natural History, Vol. 40, No. 1, p. 13; Labrador south to Connecticut, 1-80 fms.

*Tonicella rubra*

THIELE (1893) Das Gebiss der Schnecken, Bd. 2, Lfg. 8, S. 390, Taf. 32, Fig. 4.

— (1928) Fauna Arctica, Bd. 5, Nr. 2, S. 564; Helgoland, Port Wladimir.

ALLEN (1931) Plymouth Marine Fauna, 2nd ed., p. 236; New Grounds, Cawasand Bay, Wembury Bay, between tide-marks.

WINKWORTH (1932) Journal of Conchology, Vol. 19, No. 7, p. 218.

*Tonicella ruber*

SYKES (1894) Proceedings of the Malacological Society of London, Vol. 1, p. 36.

BALCH, F. N. (1906) Nautilus, Vol. 20, p. 62.

THIELE (1910) Zoologica, Bd. 22, Heft 56, S. 107.

BERRY (1917) Proceedings of the Californian Academy of Sciences, Ser. 4, Vol. 7, No. 11, pp. 231; Forrester Island, southeastern Alaska, 15-30 fms.

— (1927) Proceedings of the Malacological Society of London, Vol. 17, part 4, p. 160; Departure Bay, British Columbia.

*Chiton cinereus*

FABRICIUS (1780) Fauna Grönlandica, p. 423.

DILLWYN (1817) Catalogue of Recent Shells, p. 12.

LAMARCK (1836) Histoire Naturelle des Animaux sans Vertèbres, II édition, Tome 7, p. 505; les mers du nord de l'Europe.

*Chiton minimus*

SPENGLER (1797) Skrivter af Naturhistorie-Selskabet, Vol. 4, p. 1.

*Chiton laevis*

LOVÉN (1846) Index Molluscorum Lit. Scand., p. 28.

— (1846) Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar, Vol. 2, p. 160.

PENNANT (1877) British Zoology, Ed. 4, Vol. 4, p. 72, pl. 36, fig. 3.

*Chiton latus*

LEACH (1852) Molluscorum Britanninae Synopsis, p. 231.

*Chiton puniceus*

GOULD (1846) Otia Conchologica, p. 5.

*Chiton incarnatus*

REEVE (1848) Conchologia Iconica, Vol. 4, fig. 194.

*Tonicella squamigera*

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 18, Taf. 2, Fig. 44 50; Hakodate.

Body of medium size, oblong in outline; shell solid, elevated, sub-carinated at the back; girdle narrow, covered with minute elongate scales.

Head valve twice as long, crescentic in outline, with somewhat prominent apex; tegmental surface apparently smooth except for well-marked growth lines, under the microscope an excessively fine reticulation or series of minute granules visible; insertion plate having 8 to 11 slits; teeth sharp, smooth.

Median valves oblong, highly elevated, slightly keeled at the back, somewhat beaked; lateral slope a little concave; lateral areas slightly raised, having stronger concentric wrinkles than the central areas, ornamented likewise the head valve on the tegmental surface; jugum not defined; one slit on either side; sutural plate wide, smooth with sharp edge; sinus deep, narrow, angular.

Tail valve oval in outline with a sinuation at the middle of the anterior edge, mucro median, obtuse; posterior slope nearly straight, ornamented with minute granules like the head valve, slits 7-11 in number.

Colouration of valves very variable, usually light buff, marbled all over with orange-red in various patterns, or entirely suffused with reddish or whitish like *Tonicella marmorea* but the valves usually having a red dorsal stripe, bordered uniformly with dark red or nearly pure white; interior of the valves bright pink.

Girdle farinacious, generally reddish-brown, dusted with alternate red and whitish patches, closely covered with minute scales, which are pyriform in shape, small at the base, obtuse at the tip, curved slightly inward with smooth surface, length more than twice the width, their cross sections oval in outline; long spines sparsely distributed among these scales, holding a small spinules at the tip; hyponotum scales very minute, hyaline, truncated at the base, pointed at the tip, faintly striped near the tip, growing longer toward the margin: marginal spicules long, lanceolate with

a chitinous base, marked with two sets of oblique striations.

Gills occupying the posterior half of the body, ctenidia 7 to 15 on each side.

*Measurements :*

BODY		VALVE				NUMBER OF GILL	LOCALITY
LENGTH	WIDTH	HEAD	4TH	TAIL	DIVER- GENCE		
5.7mm	3.45mm	2.1mm	2.7mm	1.8mm	95°	7	Mutsu Bay
7.0	3.80	2.3	3.0	1.8	98°	10	
—	—	2.3	3.0	1.8	96°	—	
12.0	5.00	2.9	3.4	2.15	110°	—	Okusiri Id.
—	—	3.0	4.2	3.1	112°	—	Lubec, Me., U. S. A.
—	—	3.8	4.6	3.3	114°	—	
10.0	6.7	4.0	4.9	3.2	110°	12	Strömsund, Sweden
15.0	8.0	4.2	5.2	3.6	111°	—	
10.5	6.0	4.2	5.0	3.3	115°	12	Eastport, Me., U. S. A.
14.0	8.0	4.5	6.2	4.0	123°	15	
—	—	4.5	5.2	3.3	114°	—	Lubec
14.0	8.0	4.6	6.0	4.2	125°	14	Eastport
14.0	8.5	4.7	6.4	4.3	123°	13	
13.0	8.0	4.7	6.4	4.5	125°	13	
—	—	5.0	6.0	4.0	118°	—	Lubec
—	—	5.0	6.2	4.0	118°	—	
17.0	10.0	5.2	7.4	4.5	117°	—	Eastport
—	—	5.3	7.0	4.5	123°	12	
17.0	10.0	5.4	7.3	4.6	120°	15	
—	—	5.5	7.2	5.0	124°	14	
18.0	10.0	5.5	7.8	5.0	122°	14	
17.0	10.0	6.0	7.5	5.0	121°	—	
—	—	6.0	7.8	—	122°	—	North Pacific
—	—	6.15	8.0	5.3	127°	—	Eastport

BODY		VALVE				NUMBER OF GILL	LOCALITY
LENGTH	WIDTH	HEAD	4TH	TAIL	DIVER- GENCY		
—	—	6.2mm	8.3mm	5.5mm	125°	—	Eastport
19.0mm	12.0mm	6.25	8.2	5.8	120°	13	
—	—	6.5	9.0	6.0	127°	—	
—	—	6.5	9.8	7.0	123°	—	North Pacific
—	—	6.6	9.8	7.0	134°	—	
20.0	12.0	—	—	—	—	—	(fide PILSBRY)
25.0	—	—	—	—	—	—	Alaska (PILSBRY)

Remarks: THIELE described a small species, *Tonicella squamigera*, collected from Hakodate by HILGENDORF, which is, according to him, easily distinguished from *T. ruber* in having more highly elevated shells, longitudinal wrinkles in the central area, oval scales and simple small spicules in the girdle, short pyriform central tooth and the peculiarly shaped centro-lateral tooth.

With careful comparison of the small specimens collected from Mutsu Bay and Hokkaidô, with the typical forms in my hand of *T. ruber* from Europe and North America there seems no specific distinction between these two as is shown below.

The tegmental surface of the central area of *T. ruber* is finely reticulated, while that of *T. squamigera* is wrinkled, in which the minute round pustules coalesce to form somewhat oblique lines in two different directions, that are crossing one another so as to give an appearance of a reticulation, and as it grows larger they are worn out leaving only the skeletal outline of reticulation, so that these are not essential but of subordinate features in respect to structure of the tegmental surface. Furthermore the valves are, as in other species of the genus, relatively higher in the young than in the old ones, that is to say, the shells become flatter as they grow older the divergency ranging from 110° to 130°.

The evidence afforded by *T. squamigera* is not strong enough to dissociate as distinct species. The difference in size as well as in shape in the girdle scales between these two forms is to be attributed to different stages of growth, because the characteristic scales of *T. squamigera* gradually pass over continuously to the other. The central tooth is pyriform in outline in an apical view, while it is elongate-oblong in a front view,

THIELE pointed out such differences as the distinguishing characters of these two species. Moreover the centro-lateral tooth of the two forms agrees completely with each other. Therefore it seems natural to treat *T. squamigera* as a synonym of *T. ruber*.

Locality: Off Imabetu, 2 specimens collected by S. HÔZAWA in July, 1929.

Distribution:

Atlantic Ocean; Norway, Sweden, England, North Sea, Greenland, Labrador south to Connecticut.

Arctic Sea; Russian Lappland,

Pacific Ocean;

Bering Sea, Alaska; Attu, Sitka, Forrester Island.

North America; Monterey Bay, California.

Kamchatka; Japan; Okusiri, Hokkaidô, Mutsu Bay.

##### 5. *Tonicella submarmorea* (MIDDENDORFF, 1846)

Pl. XIV, fig. 16; Pl. XVIII, figs. 6-8; Pl. XIX, figs. 1, 3-8.

###### *Chiton submarmoreus*

MIDDENDORFF (1846) Bulletin de la Classe Physico-Mathématique de l'Académie de Saint-Petersbourg, Tome 6, No. 8; Tugurbusen; Schantarische Insel, süd Ochotskische Meer.

SCHRENCK (1867) Reisen und Forschungen im Amur-Lande, Bd. 2, S. 276-278; Castries Bay; Hakodate Bay, 20-38 feet.

###### *Chiton (Platysmus) submarmoreus*

MIDDENDORFF (1847) Mémoires de l'Académie Impériale des Sciences de Saint-Petersbourg, Ser. 6, Tome 6, pp. 82, 90, 162; (Malacozologia Rossica, pp. 18, 26, 98); Tugurbusen.

###### *Chiton (Stenosemus) submarmoreus*

MIDDENDORFF (1851) Reisen in den Norden und Osten Sibiriens, Bd. 2, Theil 1, S. 178-183, Tab. 14, Fig. 7-10; Tab. 15, Fig. 7, 8; Sitcha

###### *Tonicella submarmorea*

DALL (1878) Proceedings of the United States National Museum, Vol. 1, pp. 296, 327.

— (1878) United States National Museum, Bulletin 1, pp. 78, 109, pl. 1, fig. 7; The Aleutian Islands to Sitka and Juan de Fuca Straits.

— (1886) Proceedings of the United States National Museum, Vol. 9, p. 210.

PILSBRY (1893) Manual of Conchology, Vol. 14, p. 42, pl. 10, figs. 16-24.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 113.

BERRY (1917) Proceedings of the Californian Academy of Sciences, Ser. 4, Vol. 7, No. 10, p. 234.

— (1917) Proceedings of the United States National Museum, Vol. 54, No. 2223, p. 3.

###### *Lepidochitona (Tonicella) submarmorea*

DALL (1921) Smithsonian Institution United States National Museum, Bulletin No. 112, p. 188.

###### *Catharina submarmorea*

DUNKER (1882) Index Molluscorum Maris Japonici, p. 159.

*Chiton insignis*

REEVE (1848) Conchologia Iconica, Monograph of the genus *Chiton*, Vol. 4, pl. 22, fig. 148, species 148.

*Chiton sitchensis*

MIDDENDORFF (1846) Bulletin de la Classe Physico-Mathématique l'Académie de Saint-Petersbourg, Tome 6, p. 121.

*Chiton (Stenosemus) sitchensis*

MIDDENDORFF (1847) Mémoires de l'Académie Impériale des Sciences de Saint-Petersbourg, Sér. 6, Tome 6, pp. 72, 87, 90, 176, Malacozootologia Rossica, pp. 8, 23, 26, 112; Taf. 13, Figs. 1, 2.

*Tonicia sitchensis*

DUNKER (1882) Index Molluscorum Maris Japonici, p. 157.

*Tonicella sitkensis*

PILSBRY (1892) Manual of Conchology, Vol. 14, p. 44, pl. 11, figs. 29-31.

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 18-19.

*Lepidochitona (Tonicella) sitkensis*

DALL (1921) United States National Museum, Bulletin No. 112, p. 188.

Body of small to medium size, oval in outline; shell rather depressed with subcarinated back; girdle narrow, apparently smooth, yellowish-brown in colour, usually tessellated with dark brown.

Head valve crescentic; apex low; tegmental surface provided with many excessively minute granules which are regularly and closely arranged in a series in two different directions and crossed diagonally with each other so as to form a fine reticulation; interior of the valve smooth, polished; slit lines not grooved with many round large pores; eaves short, porous; teeth thick, short, sharp, 5 in number.

Median valve oblong in outline, subcarinated at the back, posterior edge well beaked, anterior edge straight; lateral area scarcely distinguishable, finely granulated like the head valve; central area provided with fine pustules which are smaller and more sparsely scattered than that of the lateral area; sutural laminae thick, broad and round; sinus wide, angular; interior of the valve shining, callus-ridge thick and prominent; jugal area marked irregularly with several transverse short grooves; slit line similar to that of the head valve; slit deeply incised, one on each side; teeth short, sharp.

Tail valve small, oval shaped, mucro central; posterior slope nearly straight, sculptured like the head valve, central area with similar sculpture to that of the median valve, marked with distinct concentric lines of growth; interior of valve smooth with large slit-pores along slit lines; slits 5 in number.

Tegmental surface variously coloured, though usually rosy or yellowish-



white, closely painted with spots and flames of red colour, reduced in tone toward the periphery to reddish-white; divergency  $115^{\circ}$  to  $130^{\circ}$  in adult specimens.

Girdle apparently leathery, though under the microscope the entire surface is covered densely with very fine scales, which are pyriform, longer than wide, bluntly pointed at the tip, small at the base, yellowish-brown in colour; a few remarkably long, hyaline spines distributed sparsely among these scales; hyponotum scales hyaline, exceedingly minute, elongate conical in shape, rather pointed at the tip, with fine striations on the surface, growing longer toward the periphery; margin closely beset with brown long spines and large hyaline spicules.

Radula; central tooth elongate oblong, slightly dilated and angular at the anterior end, weakly sinuated at the middle of the tip, a little constricted near the base, cusp distinct, wide and sharp; centro-lateral squarish, strongly expanded at the outer edge, with a slight sinuation at the anterior margin, inner edge smooth, nearly straight, basal plate oblong, distinct; major lateral tricuspid, its inner cusp smallest and sharpest, middle one truncated at the edge, outer one as large as half the whole cusp; shaft thick, stout, having a small, half-round wing at the anterior angle

*Measurements:*

BODY		VALVE				GILL	LOCALITY
LENGTH	BREADTH	HEAD	4TH	TAIL	DIVERG- ENCY		
—	—	2.5 mm	3.0 mm	2.2 mm	$104^{\circ}$	—	Mutsu Bay
—	—	2.6	3.1	2.1	$113^{\circ}$	—	Okusiri Id.
—	—	2.8	3.2	2.1	$108^{\circ}$	—	
23 mm	14 mm	6.5	8.5	6.3	$138^{\circ}$	23	Akkesi
22	12	6.8	8.5	6.3	$132^{\circ}$	24	Aniwa Bay
—	—	7.3	10.2	6.0	$130^{\circ}$	—	Akkesi
—	—	8.0	10.6	7.0	$140^{\circ}$	—	Aniwa Bay
—	—	8.2	11.4	7.8	$136^{\circ}$	22	
—	—	9.0	11.2	7.0	$133^{\circ}$	—	
10	6	—	—	—	$130^{\circ}$	—	Sitka
38	24	—	—	—	$115^{\circ}$ – $130^{\circ}$	24	Okhotsk Sea

of the inner edge and smooth, nearly straight at the outer edge; inner small-lateral with oblong basal plate and a bifurcated strong ridges, protruding forward at the tip; major uncinus shaped like a spoon with fine, many regularly arranged cusps at the anterior edge, with a thick, stout shaft upon a small basal plate; inner marginal somewhat irregular oblong shape with a large process at about the middle; median marginal elongate octangular in outline; outer marginal squarish as broad as wide, with deep insertion groove at the inner edge.

Ctenidium: Merobranchial abanal, extending from the 3rd to the 7th valves and consisting of 22 to 24 gills on one side.

Remarks: The present species bears a much resemblance to *Tonicella marmorea*. Though it is distinguished from the latter by its more depressed and less carinated median valve, different colour-pattern on the tegmentum, much longer marginal spines and broad wing of the centro-lateral tooth.

Of these distinguishing characters, the divergency of valves, as already pointed out by SCHRENCK (1867), is not constant and does not always tally with the original values given by MIDDENDORFF, who seemed to be of the opinion that this is the most important feature in separating this species from *T. marmorea*. As a matter of fact the divergency is usually about 100° in young specimens and becomes larger as they grow older. Therefore the character like this is not peculiar to these two forms and does not seem to be an absolute distinctive feature.

Sometimes it is difficult to differentiate this species from *T. lineata*, as the shells are similarly coloured. Even in such a case its microscopical granulation on the tegmental surface serves very well as one of the most distinguishing characters.

*T. sitchensis* was described by MIDDENDORFF from a single specimen, and has not been found by any later collector. THIELE (1909) examined the type specimen of this species, belonging to the Petersburg Museum, and considered that it should be a small specimen of *T. submarmorea*. The results worked out by THIELE on the radula agree well with the preparations before me.

Locality: Off Kanida, one small specimen was collected by S. TAKA-TUKI in July, 1927 (specimen number 1716).

**Distribution:**

The Bering Islands (DALL); Nicolski, between tide-marks (BERRY).

The Aleutian Island (DALL).

Alaska; Sitka (WOSNESSENSKI coll.); Juan de Fuca Straits (DALL,

PILSBRY); Forrester Island, 15–25 fathoms, (BERRY).

U. S. A.; Puget Sound (DALL).

Okhotsk Sea; Tugurbusen, Schantarischen Inseln (MIDDENDORFF): Bay of Castries, 20–38 feet (SCHRENCK).

Japan; Saghlien; Aniwa Bay (T. URITA)

Hokkaidô; Akkesi (HADA and OKUDA coll.); Hakodate (collected by LINDHOLM, reported by SCHRENCK); Okusiri Island (T. KINOSITA coll.)

Honsyû; Mutsu Bay.

This species was originally described by MIDDENDORFF from the southern Okhotsk Sea and later by different authors from the Bering Sea and other various places along the Pacific coast of north America.

This is peculiar to the northern Pacific in its distribution, as will be seen from the localities shown above, ranging from the Bering Sea to Puget Sound (about 48° N.) on the eastern coast and to Mutsu Bay (41° N.) on the western side, the latter locality probably being the southern limit.

It was recorded by MIDDENDORFF from the southern Okhotsk Sea near the tide-mark and by BERRY from Bering Island between tide-marks, while SCHRENCK collected it at the depth of from 6 to 12 m at the Bay of Castries and never found it at the depth more than 12 m, and from Forrester Island BERRY described it from the waters 27 to 45 m deep.

The young individuals always live deeper than 50 m and they seem to come up gradually into the shallow water as far as the tide-mark as they grow larger.

### Subfamily B CALLOCHITONINAE

#### Genus SPONGIORADSIA PILSBRY 1893

#### 6. *Spongioradsia foveolata*, nov. sp.

Pl. XIV, fig. 4; Pl. XX, figs. 1–10; Pl. XXI, fig. 1.

Body of small size, 6 mm in length, oval in outline, with well vaulted shells, dull purplish-red in colour; girdle nearly smooth, tinted with pale violet.

Head valve of crescentic shape, thick, spongy, very fragile, apex prominent, moderately raised, finely reticulated with minute elongate granules in quincunx pattern on the whole surface, punctulated by the numerous black dots of megalæsthetes near the apex; interior of the valve

nearly smooth, with the two low ridges near the posterior edge, hind parts of these two ridges rather regularly, coarsely pored; central callus much thickened posteriorly, having many transversely elongate pores; slit-rays never grooved, provided with a few large pores in rather irregular arrangement; insertion plate thick; teeth 15 in number, widely separated from one another, spongy in nature; eaves broad, spongy; posterior border of the tegmentum broadly reflexed.

Median valve oblong, slightly concave at the middle of the anterior edge, prominently beaked at the posterior end; surface smooth, slope nearly straight on either side, subcarinated at the ridge, lateral area not elevated, but trify distinctive by its peculiar pattern in the same manner as that of the head valve; megalæsthetes scattered quincuncially near the anterior border of this area; jugum not defined; central area broad, smooth, only roughened by several small pustules, arranged sparsely in a longitudinal series, traversed by some feeble lines of growth, foveolated near the outer corner except for the second valve, its whole surface finely woven by feeble threads in running longitudinally as well as transversely; central callus strongly developed in the middle of the interior of the valve, strikingly marked by a short groove at its front boundary; another callus distinct, bounding the anterior border of the lateral region with a shallow groove of the slit-rays in front of it; small area behind this callus provided with many, large, rather regularly arranged pores; two deep slits of the insertion plate widely separating teeth one from another; posterior border broadly reflexed; sutural laminae low, wide with round edge, continuous with each other; jugal sinus indistinct, very flat and narrow, divergency  $130^{\circ}$ .

Tail valve about semicircular in outline, its anterior edge nearly straight, but protruded a little at the middle; mucro prominent, not so much raised, directed anteriorly, situated at about the anterior third of the tegmentum, anterior area sculptured like a central area of the median valves; posterior slope straight, ornamented with elongate granules and black dots of megalæsthetes like the head valve. Mucronal region in the interior of the valve hollowed, with many pores in concentric arrangement; slit-rays shallowly grooved, provided several large pores; insertion plate thick, well developed, though very fragile, having 13 slits; sutural laminae low and flat, truncated at the anterior edge, connected with each other by a lamina, with a shallow sinus between them.

Tegmental surface of head valve, lateral areas of the median valves and posterior area of the tail valve equally tinted with flesh-pink; central

area marbled with longitudinal irregular lines of coral red on the ground colour of light-yellow; interior of valves all pinkish; perinotum of the girdle light-purplish-vanaceous or pale purple-drab in colour.

Perinotum of the girdle covered densely with long, sharply pointed spines of various length, brownish in colour with smooth surface, protruding at the periphery; hyponotum clothed with exceedingly minute scales of conic shape, obtusely pointed at the tip, strongly ridged in a longitudinal series of granules, standing upon the squarish, flat, thin basal plate with a shallow notch at the inner edge; small hyaline spinules seen at the periphery, distinctly striped with a few longitudinal lines.

Central tooth of radula oblong shaped, constricted a little near the tip, protruding at the anterior end, cusp with entire edge, base truncated, basal plate elongated triangular in shape; centro-lateral having a broad extension at the outer edge, slightly indented at the outer corner, somewhat sinuous at the anterior edge, inner edge almost straight with oblong basal plate; major lateral stout, tricuspidate, median cusp the strongest, shaft thickened at the tip with pointed process at the middle on the outside; inner small-lateral broad, delicate with low oblique ridge; major uncinus spoon shaped, cusped entirely, with stout, thick shaft on small basal plate; inner marginal elongate oblong, with well protruded central process; median marginal similarly shaped to the preceding one; outer marginal nearly squarish, truncated at the outer and the posterior edges.

Remarks: This species resembles much *Callochiton larvis* of Europe in many respects of the shell characters, in the girdle armatures and even in the colouration, though the latter has much more solid shells, more numerous slits in the insertion plate of the median valves, the distinctly raised lateral areas and sharply keeled shells.

It seems to me that *Spongioradsia aleutica* and *S. multidentata* are intimately related with each other in having very spongy eaves, two slits on each side of the median valves, the less elevated lateral area, the continuous sutural laminae, megal aesthetes on the surface of the valve and in small body size, while the present species is distinctive from the latter two in such characters as its well developed teeth of the valves, distinctly beaked median valves, position of the mucro in the tail valve, smoothness of girdle spines.

It seems to be natural to classify this species with the genus *Spongioradsia*, for the much fragile nature of the shell, very spongy eaves, number of slits of the median valves, though, it has, as noted above, some relationships to the genus *Callochiton*.

*Spongioradsia* was proposed by PILSBRY (1894) with the type of DALL's species *Trachyradsia aleutica* as a subgenus of the genus *Trachydermon* with some doubt in relation to the genus *Callochiton*.

THIELE (1929) ranked it as a subgenus of *Lepidochiton* under the subfamily Lepidochitoninae which is distinguished from Callochitoninae by the absence of the eyes on the shells, the separated sutural laminae and the comb-like cusp of the major uncinus.

It is easily recognized from the description of PILSBRY that the type of the group *Spongioradsia*, however, has numerous eyes on the shell and continuous sutural laminae, as he says that "the whole surface is obsoletely punctulated by the comparatively large megal aesthetes" and "the sinus, as in typical *Callochiton*, is bridged by a lamina extending across from one sutural lamina to the other". According to THIELE's system, as above mentioned, the group *Spongioradsia* is a member of the subfamily Callochitoninae, resembling *Callochiton* in having the continuous sinus, as pointed out by PILSBRY. Thus it seems to be reasonable to rank the group under the subfamily Callochitoninae.

Locality: Ōma Bay, Station 105, 1 specimen was collected by HÓZAWA, TAKATUKI and H. SATÔ in August, 1927, sp. no. 2196. Body length 6 mm.

## Family 2 MOPALIIDAE

### Genus MOPALIA GRAY 1847

#### 7. *Mopalia hirsuta*, nov. sp.

Pl. XIV, fig. 11; Pl. XXI, figs. 2, 4-6; Pl. XXII, figs. 1-6; Pl. XXIII, figs. 12, 13.

Body small, elongate-oval in outline; shell strongly elevated with subcarinated ridge, coarsely reticulated on the whole surface; girdle narrow, setose with numerous, long setae, painted generally with brownish-red; it is one of the most beautiful species in the genus.

Head valve thin, smaller than the semicircle; apex indistinct; eight radiating series of regularly increasing tubercles arranged in correspondence with the slits; posterior edge wavy, but not strongly dentated by a similar series of tubercles; entire surface between these ray-ribs rather regularly sculptured by a reticulum of pitting and netting; all sculptures become obsolete toward the apex; eaves very short; teeth long and thick; slit deep, distinct, eight in number; interior of the valve smooth, shining without a callus; slit-rays not grooved, provided with a few minute pores.

Median valves oblong in shape, straight at the anterior edge, except

for the second valve, slightly beaked at the posterior border in the middle, much elevated, subcarinated at the dorsal ridge; side-slope straight; central area coarsely reticulated by radial and oblique riblets, which become obsolete toward the jugum; lateral area distinctly raised, bordered sharply from the central area by a strong diagonal rib, formed by the partial coalescence of large, somewhat elongate tubercles, sculptured with regular pittings and tubercles, arranged almost in a checker pattern, its posterior edge a little denticulated by tubercles; interior of the valve quite smooth, shining, without any distinct callus, but jugal region striated transversely by numerous fine threads; slit-ray grooved, provided with several small pores; tooth arising from short eaves, rather thin, well defined, but not sharpened at the edge; slit deep, rather narrow, one on each side.

Tail valve small, oval shaped, shallowly sinuated at the middle on both edges; mucro at about the posterior third of the tegmentum, slightly elevated, directed forward at the tip; diagonal ribs weaker than those of the median valves; front area sculptured more coarsely with relatively larger tubercles than in the intermediate valve; posterior area small with straight slope, strongly nodulose on the whole surface; interior of the valve strongly thickened at the posterior edge, at the central and the front calli, shallowly notched on either side; slit-rays inconspicuous, accompanied by a series of 4 or 5 elongate pores; jugal region marked with numerous transverse short lines; sutural laminae separated from each other by a deep narrow sinus, extending broadly forward, truncated sharply at the front edge.

Jugal and central areas of the tegmentum striped longitudinally with broad lines of sea-shell-pink or coral-red on the ground colour of light-buff; lateral area also similarly coloured except for the third valve, which retains alone the ground colour in correspondence with the light patches of the girdle, which is generally painted by salmon-colour or light-salmon-orange; interior of the valve light-sea-shell-pink at the central area, fading toward the periphery.

Girdle rather narrow, setose, deeply slit posteriorly; perinotum covered with numerous setae of various length, arranged with such regularity that the longest situated at the sutures and the ends of terminal valves, subsidiary setae placed at about the middle between the primary ones, and subsequent ones beset successively at the intermediate position between the preceding ones. Several long, slender, curved bristles imbedded nearly in their proximal half in a conchyolinous substance of the setae in a row for a fair distance from one another, tipped by a minute calcareous

spinules at distal ends, with small roots at the base. Solitary bristles found in a group near the margin of the perinotum, apparently at a young stage judging from its similarity to the long setae. Leathery surface of the perinotum composed of exceedingly minute, long, densely crowded scales, nearly cylindrical in shape with a small tip, strongly striated by a few longitudinal ribs; marginal spicules long, hyaline, smooth or obliquely striped on the surface with pointed tip; scales of the hyponotum elongate oval in shape, considerably minute, hyaline, striated with fine, rather irregular, longitudinal lines, having a tendency to become narrower toward the periphery.

Radula: central tooth with a broad, entire cutting edge at the tip, immediately behind of which strongly constricted, dilated at the middle, and again narrowing backwardly with a truncated posterior edge; centro-lateral broadly extended outwardly so as to form a conspicuous wing at the outer margin, straightened at anterior and inner edges, alated a little at the outer corner, basal plate oblong shaped; major lateral tricuspid, with the strongest cusp at the middle, the smallest at the out side, cusps all long and sharp at the tip; shaft thick, slightly dilated at the outer margin with a small process at the anterior end, ridged remarkably in two rows at the back; major uncinus long spoon shaped, with a broad, entire, sharp cutting edge and small basal plate; inner and median marginals elongate oblong in outline; outer marginal squarish, slightly longer than wide, straight at the front as well as at the outer edge, protruding at about the middle on the inner margin.

Ctenidia merobranchial abanal extending from the third valve to the eighth valve, with 24 gills on one side.

Size and divergency; Body length 9 mm, divergency 105°.

Remarks: This species is sufficiently distinct from *Mopalia middendorffi* in many particulars as having the coarse sculpture of the tegmentum, the denticulated posterior margin of the valves, an acute divergency of the median valves, a small calcareous tip of bristles in the girdle, a minute, strongly ridged scales on the perinotum and the more elongate body, and from *Mopalia schrencki*, *M. retifera*, *M. ciliata* and *M. wosnessenski* as having coarse sculpture and reddish colouration, an acute divergency of the median valves, although much related to *M. middendorffi* in colouration, in arrangement of bristles, in shape of the scales on the perinotum and also to *M. schrencki* in structure of the bristles.

As is seen from the above remarks, this can not be referred to any of the known species of the genus, and I do not hesitate to make it a



new species.

Locality: Off Syukunobe, in the line drawn between Benten and Kurosaki, station 63 (V); 1 specimen was collected by HÔZAWA and KOKUBO on August 10, 1926; sp. no. 669.

## Genus *PLACIPHORELLA* (CARPENTER) DALL 1878

### *Placiphorella*

DALL (1878) Proceedings of the United States National Museum, Vol. 1, p. 303, 306 (CARPENTER MS.).

### *Langfordiella*

DALL (1925) Nautilus, Vol. 38, No. 3, p. 96.

## 8. *Placiphorella stimpsoni* (GOULD, 1895)

Pl. XIV, figs. 13, 17; Pl. XXI, figs. 3, 7-9; Pl. XXII, figs. 7-15.

### *Chiton (Molpalia) stimpsoni*

GOULD (1859) Proceedings of the Boston Society of Natural History, Vol. 7, p. 165.

— (1860) Otia Conchologica, p. 118; Hakodate Bay.

### *Molpalia stimpsoni*

DUNKER (1882) Index Molluscorum Maris Japonici, p. 158; Loochoo.

### *Placiphorella stimpsoni*

DALL (1886) Proceedings of the United States National Museum, Vol. 9, p. 210.

PILSBRY (1892) Manual of Conchology (1), Vol. 14, pp. 307-309, pl. 62, figs. 84-87.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 115.

PLATE (1901) Zoologische Jahrbücher, Supplement-Band 5, Fauna Chilensis, Bd. 2, S. 300-307, Taf. 13, Fig. 321-327.

NIERSTRASZ (1905) Siboga-Expeditie, Monographie 48, S. 48.

PELSENEER (1906) Mollusca in LANKESTER's Treatise on Zoology, Part 5, fig. 32 (figure after PLATE on page 49).

THIELE (1909) Zoologica, Bd. 22, Heft. 56, S. 4, 7.

BERRY (1917) Proceedings of the United States National Museum, Vol. 54, No. 2223, pp. 12-13, pl. 8, figs. 1, 2; pl. 9, figs. 1-8.

DALL (1921) Smithsonian Institution United States National Museum, Bulletin 112, p. 197; Bering Is.; Lower California.

THIELE (1929) Handbuch der systematischen Weichtierkunde, Teil 1, S. 11, Fig. 4.

KIKUTI, K. (1931) Toyamawan Nantaidôbutu Mokuroku (Catalogue of Mollusca of Toyama Bay), p. 1, no. 2.

YAGURA, W. (1932) Hyôgokensan Kairui Mokuroku (Catalogue of Mollusca of Hyôgo Prefecture), p. 20, no. 243.

TAKI, IWAO (1932) Memoirs of the College of Science, Kyoto Imperial University, Ser. B, Vol. 8, No. 1, pp. 33-42, Figs. 13-28, plate 2, figs. 4-6.

HIRASE, S. (1934) A Collection of Japanese Shells, pl. 55, fig. 2, p. 30.

KURODA, T. (1935) Miyazaki-Kensan Kairui Mokuroku (Catalogue of Mollusca of Miyazaki Prefecture), p. 39, no. 1.

TAKI, IWAO (1936) Onomiti Kinkai Nantaidôbutu Mokuroku (Catalogue of Marine

Mollusca around the Onomichi Marine Biological Station), p. 1, no. 4.

TAKI, ISAO (1936) Saitō Hō-onkai Hakubutukan Zihō (Proceedings of Saitō Hō-on-Kai Museum), No. 13, no. 10.

*Placiphorella* sp.

TAKI, ISAO (1924) Dōbutugaku Zasshi (The Zoological Magazine), Vol. 36, no. 429, pp. 286-287, 1 text-figure.

HIRASE, S. (1927) Nippon Dōbutu Zukan (Figures and Descriptions of Japanese Animals), p. 1501, fig. 2885.

YAGI, S. (1931) Ehimeken Dōbutu Si (Catalogue of Animals from Ehime Prefecture), p. 67, no. 739.

ASANO, H. (1933) Bunrui Suisan Dōbutu Zusetu (Systematic Illustrations of Freshwater and Marine Animals), p. 253, fig. 351.

NOMURA, H. and H. TUNODA (1933) Saitō Hō-on-Kai Hakubutukan Zihō, special no. 3, p. 19, no. 195.

*Langfordiella japonica* (full grown form, without tail slits)

DALL (1925) Nautilus, Vol. 38, p. 96.

TAKI, ISAO (1928) Venus, Vol. No. 1, pp. 41-42.

THIELE (1929) Handbuch der systematischen Weichtierkunde, Teil 1, S. 11.

*Placiphorella blainvillei* BRODERIP } (incorrectly referred to)

*Placiphorella petasus* ADAMS et REEVE }

THIELE (1893) Das Gebiss der Schnecken, Bd. 2, S. 397, Taf. 32, Fig. 24, 25.

Body of large to medium size, broadly ovate in outline; shell depressed, subangular at the ridge, with straight side slopes; girdle of unequal breadth, being much extended anteriorly.

Head valve thick, stout, narrowly crescentic with an indistinct apex, concentrically striated on the tegmental surface, but lacking all radiating sculpture; interior of the valve smooth, unusually thickened anteriorly; slit-lays inconspicuous; teeth remarkably thick, short, strongly pectinated irregularly; slit 8 or more.

Median valve oblong in outline, exceedingly wider than long, subcarinated at the jugum, not beaked posteriorly, almost straight at the front edge; lateral areas usually distinct, slightly elevated, bordered by inconspicuous low diagonal and sutural ribs; the space between ribs smooth, slightly concave; interior of the valve smooth, not distinctly callused; slit-lays not defined; slit narrow; teeth thick and short, obtuse at the edge, slightly pectinated on the outer surface, rising from narrow eaves; posterior edge widely reflexed; sutural laminae very wide, thick, nearly straight and sharp at the front edge; incised by a shallow, v-shaped sinus.

Tail valve small, depressed, its greatest width, including insertion and sutural-plates, measuring hardly more than half the width of the widest median valves; anterior edge regularly arched with a slight sinuation at the posterior end in the middle; mucro lies near the posterior margin;

central area occupies the greater part of the tegmentum, flat but roughened by irregular lines of growth, bordered posteriorly by fairly prominent diagonal rim in the median valves due to its inward reflexion; insertion-plate very short, rising from a heavy callous rim, having one oblique slit on each side and faint waving along the posterior margin; sutural laminae broad, smooth, flat, sufficiently expanded anteriorly, truncated at the front edge, deeply cut into two halves by a narrow sinus.

Girdle unequal in breadth around the valves, being much wider anteriorly; perinotum sparsely beset with exceedingly minute spinules which are sharply pointed and deeply striated at the tip, measuring  $30\mu$  in length; large bristles at the suture and the margin, intermingling a number of small ones; all the bristles composed of small spinules, which are pointed at the tip and with a small root at the base, light brownish in colour, densely arranged in oblique series around the axis like the arrangement of shoots of plants; marginal spines long, hyaline, sharp at the tip, faintly striped nearly throughout the entire length, with a small root at the base, measuring  $130\text{--}160\mu$  in length; hyponotum having much developed pallial fold, deeply incised at the posterior end, extending forwardly so as to form several tentacular processes, its entire surface densely covered with hyaline, long, smooth, pointed spinules, measuring  $80\text{--}100\mu$  in length, the peripheral ones being nearly equal to the marginal spines in length.

Radula typically mopaloid; central tooth oblong in outline, slightly sinuated at the middle on both sides, nearly straight at the front end with well developed cusp, trilobular at the posterior end; centro-lateral having very broad wing at the outer edge, slightly reflexed at the outer corner, weakly sinuated at the anterior edge, inner margin much curved, basal plate elongate-oblong; shaft of the major-lateral thick and stout, strongly ridged at the outer margin with a broad wing at the base, inner edge nearly straight and smooth, dorsal ridges forming two distinct processes, cusp small, cut into three denticles almost in equal size; inner small-lateral broad, very much elevated near the inner edge to articulate with the dorsal process of the major-lateral; major-uncinus spoon shaped, slightly curved at the base with a elongate basal plate, cusp arched, entire, sharp; inner-marginal elongate oval shape with a strongly developed triangular process at about the middle; median-marginal squarish in shape, slightly sinuate in front, protruding at the posterior edge.

Colouration of valve whitish along the middle, the side slopes mottled and streaked with greenish-yellow, olive and blue or olivaceous and orange-ash, thus giving a general effect of dark olivaceous; interior of the valves

bluish-green or nearly white on the whole surface.

Ctenidia holobranchial abanal, extending along the entire length of the foot, about 26 on each side; a specimen of 29 mm in length has 15 to 19 gills on one side.

### Size

BODY		SHELL		
LENGTH	BREADTH	LENGTH	BREADTH	DIVERGENCY
18 mm	14 mm	13 mm	10 mm	128°
22	18	16.5	13	137°
27	22	19	15.5	138°
33	24	24	18	138°
36	24	26	19	134°
---	29	28	23	140°
—	29	28	23	137°
42	33	30	23	136°
56	32	34	24	140°

Remarks: GOULD first described this species under the name *Chiton stimpsoni* basing on the small specimens collected by STIMPSON from Hakodate Bay at the depth of 25 fathoms. THIELE figured the radula of this species under the erroneous name *Placiphorella blainvillei* and *P. petasus*, both collected from Japan, and later corrected the mistakes in his enormous work entitled "Revision des Systems der Chitonon". PLATE (1901) gave details of the anatomical structure of this species from the material taken from Hakodate, and the excellent descriptions are given of some characters of shell by PILSBRY (1892) and that of the girdle and the radula by BERRY (1917) from the study of the alcoholic specimens collected by the "Albatross" from Hakodate.

DALL established new genus *Langfordiella* with the type *L. japonica*. Examination of the type specimen, this species agrees completely with *Placiphorella stimpsoni* in all characters except for the slits of the tail valve. The young specimens of this species have a distinct slit on each side of the tail valve. But the slit dwindles as it grows older and moreover as the surface of the insertion plates becomes much wavy so as to form an

irregular pectination, the slit disappears into the waves. PLATE (1901, p. 301) states that the slit is not always present in the tail valve of this species. Therefore the presence or absence of the slit in the tail valve loses its significance as the criterion to distinguish these two forms.

In 1926 I sent the late Dr. DALL of Smithsonian Institution specimens collected from Ehime and Kanagawa Prefectures, asking his opinion as to whether his new species might be referable to *P. stimpsoni* of GOULD.

His reply was "it is evident from the specimens that you are right in identifying your species with GOULD's *P. stimpsoni*, and in showing (which my *Langfordiella* specimens did not do) the variation in the condition of the tail valve". DALL's new form and GOULD's species can not be denied to be synonymous.

THIELE (1929) ranked *Langfordiella* as a subgenus of *Placiphorella*, although it is much more reasonable to make the former as a synonym of the latter by reason of the evidence given above.

### *Locality*

SP. NO.	STATION	LOCALITY	COLLECTOR	DATE	NUMBER OF SPECIMEN
671	74 (III)	off Karibazawa	KOKUBO and KAMADA	Aug. 22, 1926	1
677	45 (III)	off Tubakiyama	HOZAWA and TAKATUKI	Aug. 2, 1926	3
1730		off Kanida	TAKATUKI	July 23, 1927	2
1757		off Hiradate	TAKATUKI and SATO	July 24, 1927	1
2428	116	off Huzisima	HOZAWA	July 22, 1929	1
		Asamusi	Iw. TAKI	Aug. 9, 1930	1
					Total 10

### Distribution :

#### Pacific coast

Hokkaidô: Akkesi (collected by HADA and OKUDA). Hakodate (STIMPSON coll., GOULD described, PLATE, DALL, PILSBRY, DUNKER, BERRY). Tugaru Straits (PLATE, collection by the "Sôyô-maru", Station no. 654).

Honsyû: Hirotamura, Kesen-gun; Miyako; Sakiyamamura, Simohei-gun (collected by G. TOBA), Iwate-prefecture. Onagawa Bay (collection at the Onagawa Marine Laboratory), Miyagi-prefecture. Onahama (NOMURA and TUNODA; TAKI), Hukushima-prefecture. Minato

(SASAMOTO), Ibaragi-prefecture. Emi; Nemoto (DALL, LANGFORD); Hôzyô (Y. OKADA and K. BABA), Tiba-prefecture. Misaki (TAKI) Kanagawa-prefecture. Okitu (A. HUZITA coll.); Kawana, Tagatagun (Y. OKADA coll.), Sizuoka-prefecture. Seto (IWAOKI coll.); Sirasaki-mura, Hidaka-gun (K. KANDA coll.), Wakayama-prefecture. Kyûsyû: Nangô (KURODA), Miyazaki-prefecture. Tomioka, Amakusa (Y. OKADA and BABA coll.), Kumamoto-prefecture.

**Inland Sea:**

Honsyû: Awazi (YAGURA, collected by TAKASE and MORITA); Settsu; Harima (YAGURA), Hyôgo-prefecture. Onomiti (collection at the Onomiti Marine Laboratory). Hiroshima-prefecture.

Sikoku: Mitu (TAKI); Tikami (YAGI), Ehime-prefecture.

Coast of Japan Sea: Mutsu Bay, Aomori-prefecture. Sado (K. KIKUTI coll.), Niigata-prefecture. Himi; Abugasima (K. KIKUTI coll.), Toyama-prefecture. Torii-mura, Annô-gun, (TAKAGI coll.), Simane-prefecture. Hukuoka (K. BABA coll.), Hukuoka-prefecture.

**Pacific coast of North America:**

Bering Islands to Cerros Island, Lower California (DALL).

**Family 3 CRYPTOPLACIDAE**

**Subfamily A ACANTHOCHITONINAE**

**Genus ACANTHOCHITON GRAY 1821 (ACANTHOCHITONA)**

**9. *Acanthochiton rubrolineatus* (LISCHKE, 1873)**

Pl. XV, fig. 1; Pl. XXIII, figs. 1-6; Pl. XXIV, figs. 1-2; Pl. XXV, figs. 17, 18.

***Chiton rubro-lineatus***

LISCHKE (1873) *Malakozoologische Blätter*, Bd. 21, S. 24.

— (1874) *Japanische Meeres-Conchylien*, Teil 3, S. 73-74, Taf. 5, Fig. 12; Nagasaki.

***Acanthochiton rubro-lineatus***

DUNKER (1882) *Index Molluscorum Maris Japonici*, p. 160.

***Acanthochiton rubrolineatus***

THIELE (1893) *Das Gebiss der Schnecken*, Bd. 2, Lfg. 8, S. 399, Taf. 32, Fig. 32.

YAGURA (1932) *Hyôgokensan Kairui Mokuroku*, p. 20, no. 245; Awazi.

BERGENHAYN (1933) *Kungl. Svenska Vetenskapsakademiens Handlingar*, Bd. 12, No. 4, S. 45; Misaki, Sagami.

TAKI, IW. (1936) *Onomiti Kinkaisan Nantai-Dôbutu Mokuroku*, p. 1, no. 7; Onomiti.

TAKI, IS. (1936) *Saitô Hôonkai Hakubutukan Zihô*, No. 30, p. 1, no. 7; Matusima Bay; Matugahama; Kinkazan; Amizisima; Onahama; Yunosima, Mutsu Bay; Taneiti. Iwate Pref.

***Acanthochites rubrolineatus***

PILSBRY (1893) *Manual of Conchology*, Vol. 15, p. 18, pl. 2, fig. 50; Nagasaki.

PILSBRY (1895) Catalogue of the Marine Mollusks of Japan, p. 115.

NIERSTRASZ (1905) Die Chitonen der Siboga-Expedition, S. 50.

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 46, Taf. 6, Fig. 14-17; Enosima, Sagami Bai; Hakodate; Nagasaki; Tsingtau; Tschifu.

*Acanthochitona rubrolineata*

KURODA (1935) Miyazakikensan Kairui Mokuroku, p. 39, no. 3; Aosima.

*Acanthochiton zealandicus*

THIELE (1893) Das Gebiss der Schnecken, Bd. 2, Lfg. 8, S. 399, Taf. 32, Fig. 33, Nagasaki.

Body oblong-ovate in outline; medium sized; shell rather small, convex at the jugum; girdle not so much broader than median valves, beset with 18 hair-tufts, generally dark blackish-green in colour.

Head valve larger than semicircular, regularly convexed with flattened apex, slightly waved at the anterior margin; posterior edge slightly sinuated at the middle, provided with numerous, closely set, flat, oval granules; interior of the valve smooth with a well developed callus in the middle; teeth long, slightly wrinkled on the outer surface, shallowly incised by 5 small slits; posterior margin broadly reflexed.

Median valve trapezoid in shape, its breadth much broader than length, nearly straight at the anterior edge, well beaked at the posterior edge, regularly arched on both sides; jugum not sharply demarcated from pleuro-lateral areas, sculptured with fine, longitudinal wrinkles; pleuro-lateral area ornamented with numerous small, oval granules; sutural laminae rather short, extending forwardly; sinus deep, moderately wide; teeth thick, long, having a slit on each side; interior of the valve smooth, thickened at the middle.

Tail valve nearly straight at the anterior margin, vaulted at the jugum, strikingly arched at the posterior margin; mucro lies a little behind the centre; central area sculptured like that of the median valves; posterior slope somewhat convexed, granulated likewise the head valve; sutural laminae short, truncated at the anterior edge; sinus wide, flat; posterior margin of insertion plate sinuated at the middle.

Girdle covered densely with brownish smooth spinules, 20-30  $\mu$  long, intermingling with long, slightly curved, finely striped, sharply pointed spines, measuring 350  $\mu$  or more in length; hair-tufts very marked; bristles long, acute, hyaline or dark brown, over 1.5 mm in length; marginal spicules straight, nearly smooth, hyaline or brownish, somewhat longer than those of perinotum (length 430  $\mu$ ); hyponotum clothed with small, hyaline spinules, measuring 100  $\mu$  or more.

Radula: Central tooth oblong shaped, truncated at the anterior end,

serrated at the posterior end; centro-lateral square, with a small outer process at the anterior corner, a broad outer lamella at the posterior corner, weakly cusped at the anterior edge; the middle of three cusps of major lateral is the largest, its shaft stout with a short outer wing at the anterior end, a small outer process near the base; major uncinus moderately arched, with a small cusp at the front end; three marginals elongate oblong in shape; outer marginal strikingly thickened at the inner edge.

Ctenidia holobranchial with a small space between the posterior gill and the anus, ranging in correspondence with from the 4th to the tail valves, counted 21 or 22 branchiae on each side in a specimen measuring 27 mm in length.

Colouration: Shells of the type is fresh-gray coloured, maculated here and there with brown, nearly whitish on the 5th, 6th, 7th and a part of the 4th valves; much reddish on the 2nd valve; another valves are generally dark reddish-brown, having a wedge-shaped olive spot which is separated by a whitish longitudinal line in the middle of the 2nd, 3rd and 4th valves, and painted obliquely by longitudinal red lines.

There are many variegated specimens in this species, for example, the girdle is yellowish throughout with white hair-tufts, uniformly brownish, dark brownish or dark greenish, and the shells are brownish with the dark longitudinal bands on the lateropleural areas as well as at the boundaries of the jugum, freckled with dark brown on the pale ground colour, or painted thoroughly with dark green; the interior of the valves

### *Locality*

SP. NO.	STATION	LOCALITY	COLLECTOR	DATE	NUMBER OF SPECIMEN
664	26 A (I)	Hutagozima	HOZAWA and ITO	July 9, 1926	3
1885		Tutiya	TAKATUKI and SATO	July 29, 1927	3
1921	26 (II)	Hutagozima	HOZAWA and KOKUBO	Aug. 10, 1927	1
2052	102	Takaisozaki	HOZAWA and TAKATUKI	Aug. 17, 1927	1
2477	117	Tappiobisima	HOZAWA	July 22, 1929	1
—	—	Asamusi	Iw. TAKI	Aug. 7, 1930	2
—	—	Aburamegasaki	Iw. TAKI	Aug. 8, 1930	14
—	—	Asamusi	Iw. TAKI	Aug. 9, 1930	8
—	—	Mourazima	Iw. TAKI	Aug. 10, 1930	10



is pale blue and dark at the middle.

Size: length 24 mm, breadth 20 mm (LISCHKE)

„ 27 mm, „ 13 mm (MUTSU BAY)

Remarks: The specimen which had been kept in the Löbbeck Museum was lost. THIELE (1909), however, examined several specimens from Japan and single specimen, supplied by the same museum, which he considered to be the original specimen from the type locality. According to him the distinguishing characters of this species are that the median valve is much broader than long and is nearly equal in width to that of the girdle; the mucro of the tail valve is situated at about the middle, spines of the girdle are acute at the tip with fine striations over half the length.

Distribution:

Pacific coast

Hokkaidô: Muroran (1) (EMURA); Hakodate (2) (THIELE, Iw. TAKI).  
Honsyû: Taneiti (3), Miyako (4) (TOBA), Iwate Pref. Onagawa (5) (IMAI); Kinkazan (6) (TAKI); Matusima Bay (7) (TAKEWAKI), Miyagi Pref. Onahama (8) (TAKI), Hokusima Pref. Hutori (9) (HIRASE), Tiba Pref. Yokohama (10) (TAKI), Misaki (11) (TAKI, BERGENHAYN); Kamakura (12) (INAZAWA); Enosima (13) (THIELE, TAKI), Kanagawa Pref. Simoda (14) (KANEKO), Sizuoka Pref. Ôsima (15) (ÔYAMA, YAMAMURA). Toba (20) (MORITA YAMADA), Mie Pref. Seto (21) (TAKI); Kada (22) (KANDA), Wakayama Pref.

Sikoku: Kasiwazima (25) (SUGIMOTO), Kôti Pref.

Kyûsyû: Aosima (26) (KURODA), Miyazaki Pref. Natui, Sibusi Bay (27) (HARA); Kagosima Bay (28) (Iw. TAKI), Kagosima Pref.

Inland Sea:

Honsyû: Awazi (30) (YAGURA, MORITA), Hyôgo Pref. Onomiti (31) (Iw. TAKI); Uzina (32) (TAKI), Hirosima Pref.

Sikoku: Mitu (33) (TAKI), Ehime Pref.

Kyûsyû: Sirako (34) (TAKAGI), Ôita Pref.

Western coast:

Kyûsyû: Akune (35) (MITUKURI), Kagosima Pref. Hyakkanisi (36) (Iw. TAKI); Amakusa (37) (BABA), Kumamoto Pref. Nisiariie (38) (SIKI); Nagasaki (39) (LISCHKE, DUNKER, THIELE, SONEHARA); Kisyuku (40) (YOSIDA); Tomie (41) (ÔGA), Hukuezima; Sanri, Takanosima (42) (HIROYAMA); Ituhara, Tusima (43) (EGUTI); Nagasaki Pref.

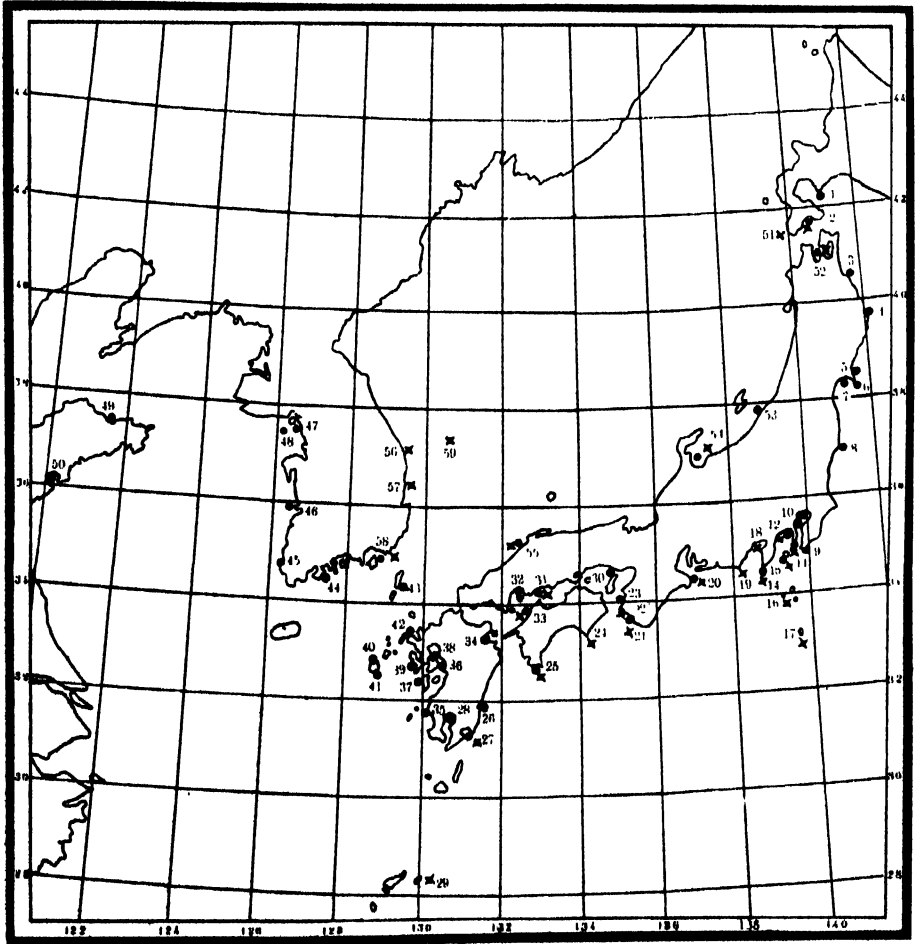
Tyôsen: Reisui (44) (OKUDA); Moppo (45) (RA); Gunzan (46) (OKUDA); Zinsen (47) (MORI, KAMITA); Tokutumisima (48) (KAMITA).

China: Tschifu (49), Tsingtao (50) (THIELE).

Japan Sea:

Honsyû: Mutsu Bay (52), Aomori Pref. Niigata (53) (EMURA),  
Niigata Pref. Toyama Bay (54) (KIKUTI), Toyama Pref. Torii-  
mura, Annôgun (55) (TAKAGI), Simane Pref.

Tyôsen (Korea): Husan (58) (YOSIDA).



Text-fig. 1. Geographical distribution of *Acanthochiton rubrolineatus* (LISCHE) (•) and *Acanthochiton achates* (GOULD) (x).

10. *Acanthochiton achates* (GOULD, 1859)

Pl. XV, fig. 2; Pl. XXIII, figs. 7-11; Pl. XXIV, figs. 3-7.

*Chiton (Acanthochaetes) achates*

GOULD (1859) Proceedings of the Boston Society of Natural History, Vol. 7, p. 165.

— (1862) Otia Conchologica, p. 118; Kikaia and Hakodate Bay.

*Acanthochiton achates*

DUNKER (1882) Index Molluscorum Maris Japonici, p. 160.

*Acanthochites achates*

PILSBRY (1893) Manual of Conchology, Vol. 15, pp. 18-19.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 115.

NIERSTRASZ (1905) Die Chitonen der Siboga-Expedition, S. 59.

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 46-47, Taf. 6, Fig. 18-23; Hakodate.

*Acanthochites subachates* PILSBRY, MS.

TAKI, Is. (1923) Dôbutugaku Zasshi, Vol. 36, pp. 288-289, 1 text-fig.; Misaki, Sagami.

BABA (1929) Dôbutugaku Zasshi, Vol. 41, p. 112, pl. 1, fig. 1. D.

*Acanthochiton sagamicus*

BERGENHAYN (1933) Kungl. Svenska Vetenskapsakademiens Handlingar, Bd. 12, No. 4, S. 43-45, Taf. 1, Fig. 14, Text-fig. 14; Misaki, Sagami.

Shell narrow, elliptical in outline; girdle wide, provided with short, unequal spines and bunches of spicules.

Head valve semioval, finely granulated on the surface, nearly straight at the posterior edge; teeth thick, long, sharp, roughened on the outer surface; slit deep, 5 in number.

Median valve trapezoid or scale-shaped, strongly beaked; jugum not so distinctly bordered, weakly provided with longitudinal striation which give a moniliform appearance near the boundaries on both sides; lateropleural area finely ornamented with elongate oval granules; sutural laminae widely protruding anteriorly; sinus deep with rounded base.

Tail valve very small compared with the size of the body, elliptical in shape, exceedingly broader than long, nearly straight at the anterior edge; mucro flat, subterminal, posterior area small, steep; posterior margin of the articulamentum straight; sutural laminae large, strikingly extending anteriorly with truncated edge in front.

Girdle covered densely with large and small spines, large ones spindle shaped, hyaline or yellowish brown, sometimes blue or bluish green in colour, conspicuously striated near the tip, with chitinous cup at the base, measuring 420-500  $\mu$  in length; small spines smooth, sharply pointed at the tip, hyaline or yellowish brown, being 70-120  $\mu$  long; marginal spines straight, nearly smooth, usually hyaline, pointed at the tip with chitinous cup at the base, attaining 550  $\mu$  in length; spinules of hyponotum somewhat flattened spindle shape, hyaline, measured 60-120  $\mu$  in length; spicules

of hair-tuft thin, long, acute at the tip, hyaline or yellowish or bluish in colour, 1.2–1.8 mm in length.

Colouration: shell generally dark brown or sooty in colour; median valve having usually 1 or 3 whitish or yellowish streaks on the jugal tract, sometimes provided with small white freckles on the lateral regions; interior of valves glaucous, sometimes reddish or brownish at the middle; girdle usually dark brown or dark blue, sometimes variegated; hair-tufts white or yellowish or brownish in colour.

Radula: central tooth oblong, slightly constricted at the middle on both sides, roundly arched and slightly cusped at the anterior edge, somewhat pointed at the base; centro-lateral squarish, having small outer process at the anterior corner, exceedingly broad outer lamella and small cusp at the anterior edge; major lateral tricuspid, its shaft thick, with rounded inner wing at the anterior end, outer wing very small; major uncinus long, stout with small cusp and large basal plate; outer marginal oblong, thickened at the inner margin.

Ctenidia holobranchial with a small space between the gill and the anus, extending in correspondence with from the 3rd valve to the tail valve or occupying 5/6 the entire length of the foot, 30 gills on each side in the specimen measuring 27 mm in length, 35 in the specimen 30 mm long.

Size: length 22 mm, breadth 12 mm

„ 27 mm, „ 15 mm

„ 30 mm, „ 20 mm

„ 31 mm, „ 17 mm

„ 32 mm, „ 20 mm

Remarks: This species bears so strong a resemblance to *A. rubro-lineatus* that even a sensible taxonomist sometimes fails to distinguish them. The following features may be a helpful guide to the clear distinction between them. In the present species, 1) the tegmental granulations are usually finer; 2) the median valves are much more beaked posteriorly; 3) the sutural laminae are longer and more protruded anteriorly; 4) the tegmental area of the tail valve is much smaller with a subterminal mucro; 5) spines of girdle are much thicker and longer, with distinct striations near the tip.

BERGENHAYN (1933) described *Acanthochiton sagamicus* from Misaki, Kanagawa Prefecture and myself (1923) reported a species under the manuscript name "*Acanthochites subachates*" from the same locality. Both forms would fall into *A. achates* of GOULD in all respects.

Locality: 5 specimens were collected by Iw. TAKI at Mourazima on August 10, 1930.

Distribution:

Pacific coast

Hokkaidô: Hakodate (2) (GOULD, THIELE).

Honsyû: Miyako (4) (TOBA), Iwate Pref. Tôkyô Bay (10) (TAKI), Tôkyô Pref. Zyôgasima (11) (TAKI), Misaki (11) (TAKI, BERGEN-HAYN), Kamakura (12) (TAKI), Enosima (13) (TAKI), Kanagawa Pref. Simoda (14) (KANEKO); Okitu (18) (A. HUZITA); Omaezaki (19) (A. HUZITA); Sizuoka Pref., Ôsima (15) (YAMAMURA); Kamizusima (16) (MIYAZI); Hatizyôzima (17) (T. HUZITA), Izu Sîtîtô. Toba (20) (YAMADA), Mie Pref. Seto (21) (TAKI); Gobô (22) (OKAMOTO), Wakayama Pref.

Sikoku: Murotozaki (24) (HIRO); Kasiwazima (25) (SUGIMOTO), Kôti Pref.

Kyûsyû: Natui, Sibusi Bay (27) (MITUKURI).

Amami Syotô: Kikaizima? (29) (GOULD).

Inland Sea:

Honsyû: Onomiti (31) (TAKI), Hirosima Pref.

Sikoku: Mitu (33) (TAKI), Ehime Pref.

Kyûsyû: Sirako (34) (K. TAKAGI), Ôita Pref.

Japan Sea:

Hokkaidô: Hukuyama (51) (T. KINOSITA).

Honsyû: Mutsu Bay (52), Aomori Pref. Toyama Bay (54) (KIKUTI).

Tyôsen (Korea): Urutin (56) (TODA), Kôgendô; Kyûryûho (57) (HATAKEDA), Keisyôhokudô; Husan (58) (YOSIDA), Keisyônandô; Utureyôtô (59) (SUGIYAMA).

## Subfamily B CRYPTOPLACINAE

### Genus CRYPTOPLAX BLAINVILLE 1818

#### 11. *Cryptoplax japonica* PILSBRY 1901

Pl. XIV, fig. 15; Pl. XXIV, figs. 8, 9; Pl. XXV, figs. 1-5, 19-21.

? *Chitonellus fasciatus*

TAPPARONE-CANEFRI (1874) Zoologia del Viaggio Intorno al Globo della Regia Fregata Magenta, Malacologia, p. 178; Japan.

*Chitonellus larvæformis*

THIELE (1893) Das Gebias der Schnecken, S. 400, Taf. 32, Fig. 36; Hakodate (HILGEN-DORF).

*Cryptoplax japonicus*

- PILSBRY (1901) Proceedings of the Academy of Natural Sciences of Philadelphia, Vol. 53, p. 204; Hirado (HIRASE).
- HIRASE, Y. (1907) Catalogue of Marine Shells of Japan, p. 12, no. 1177 a; Hirado, Hizen.
- TAKI, IS. (1924) Dōbutugaku Zasshi, Vol. 36, No. 429, pp. 287-288, 1 text-fig; Misaki, Sagami.
- (1929) Venus, Vol. 1, pp. 108, 116, fig. 54.
- BABA, K. (1929) Dōbutugaku Zasshi, Vol. 41, No. 485, pp. 112-114, text-figs. III, 1-5.
- KUSE, Y. (1930) Tatugahama Kinkaisan Kairui Mokuroku, p. 12, no. 181.
- GISIÉN, T. (1931) Journal of the Faculty of Science Imperial University of Tokyo, Sect. 4, Vol. 2, Part 4, pp. 433, 441; Misaki.
- BERGENHAYN (1933) Kungl. Svenska Vetenskapsakademiens Handlingar, Bd. 12, No. 4, S. 52-54, Taf. 1, Fig. 19, Text-fig. 17 a, b, d-f; Misaki, Sagami.

*Cryptoplax japonica*

- THIELE (1909) Zoologica, Bd. 22, Heft 56. S. 4, 8, 54-55, Taf. 6, Fig. 95-96; Hakodate (HILGENDORF).
- HIRASE, Y. (1910) Nippon Senkai Mokuroku, p. 23, no. 497; Hizen.
- TAKI, IS. and Iw. TAKI (1930) Venus, Vol. 2, No. 3, pp. 101, 103.
- YAGI, S. (1931) Ehimeken Dōbutusi, p. 67, no. 729; Tikami.
- KIKUTI, K. (1931) Toyamawan Nantaidōbutu Mokuroku, p. 2, no. 4; Takosima; Noto.
- HIRASE, S. (1934) Collection of Japanese Shells, p. 55, fig. 4.
- KURODA, T. (1935) Miyazakikensan Kairui Mokuroku, p. 39, no. 4; Aosima.
- TAKI, Iw. (1936) Onomiti Kinkaisan Nantaidōbutu Mokuroku, p. 1, no. 8.

*Cryptoplax rhodoplax*

- PILSBRY (1901) Proceedings of the Academy of Natural Sciences of Philadelphia, Vol. 53, p. 20; Hirado (HIRASE).
- HIRASE, Y. (1907) Catalogue of Marine Shells of Japan, p. 12, no. 1177 b; Hirado, Hizen.
- (1915) Conchological Magazine, Vol. 4, pl. 1, fig. 6.
- THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 55, Taf. 6, Fig. 90-94; Hirado (HIRASE); Hakodate (HILGENDORF); Enoshima (DÖDERLEIN); Nagasaki (BUNGE).
- YAGURA (1916) Hyōgokensan Kairui Mokuroku, p. 2, no. 716, Awazi.
- YAGI, S. (1931) Ehimeken Dōbutusi, p. 67, no. 730; Tikami.
- YOKOYAMA (1931) Catalogue of Marine, Freshwater and Land Shells of Japan, p. 16, no. 467; Iyo.

*Cryptoplax japonica rhodoplax*

- KANAMARU (1932) Venus, Vol. 3, No. 5, p. 237, no. 44; Simoda.

Body large, elongate vermiform, nearly equal in breadth throughout the entire length; four anterior valves usually, sometimes three anterior only imbricated, four posterior separated each other with short intervals, of which the spacing between the 5th and the 6th is the longest, between the 7th and the tail valve the shortest; girdle wide, brownish yellow in colour, banded transversely with dark brown.

Head valve: tegmentum somewhat longer than wide, ornamented with elongate tubercles, arranged densely in radial series, sometimes traversed by some lines of growth; teeth long, thick, smooth; slit deeply incised at

the front edge, 3 in number, interior of valve smooth, without slit lines.

Median valve: second valve oval shaped, shorter than tail valve, jugum defined, dilated and protruded anteriorly, sculptured with fine longitudinal lines; fourth valve having the tegmentum of about equal breadth to the second, being not so much protruded as the preceding one; latero-pleural area small, provided with 4 or 5 longitudinal series of tubercles, forming sometimes coarse riblets by their coalescence; interior of valve smooth; posterior edge strongly reflexed; sutural laminae small, protruded forward, separated by flat sinus.

Tail valve becoming narrower anteriorly, rounded posteriorly; mucro terminal, flat, blunt, inconspicuous, especially not protruded posteriorly in young specimens, but somewhat prominent in full grown ones; insertion plate short, thick, nearly vertical.

Girdle: perinotum clothed densely with spines of various size, brownish or hyaline, of which the larger ones slightly curved, smooth, sharply pointed at the tip, standing on small chitinous base, the smaller ones slightly curved or nearly straight, deeply grooved, moderately pointed at the tip; marginal spines much longer than those of perinotum, hyaline, smooth with acute tip; hyponotum densely crowded by small, curved, acute, hyaline spinules.

Colouration: Body coloured with brownish throughout, but usually tessellated rather irregularly with dark brown; tegmentum with dark brown; articulamentum whitish, bluish, greenish or bright rose coloured.

Radula: central tooth oblong shaped, rounded anteriorly, with strong cusp at the tip, slightly waved on both sides, bilobed at the posterior end; centro-lateral having broad outer wing at the posterior corner, notched at the anterior corner, nearly straight at the front edge; major lateral tricuspid, the median cusp is the longest, shaft nearly straight in both edges, with small wing at the anterior corner, strongly ribbed at the back; major uncinus long oar-shaped, subtruncated at the anterior end with small cusp; base large thick; outer marginal nearly square.

Gill: merobranchial, branchiae about 18 on each side, disposed in correspondence with the interval between the 7th and the 8th valve.

Size: The type specimen measured 28 mm in length in dried condition; body 57 mm in length (BERGENHAYN); the specimens of this species taken from Mutsu Bay are all very small and most of them are strongly curled; two uncurled specimens measured respectively 20 mm and 30 mm in length.

Remarks: The species which was reported by TAPPARONE-CANEFRI

(1874) from Japanese waters may be referred in all probability to PILSBRY's species.

THIELE (1893) figured and described the radula of this species as of *Chitonellus larvaeformis* and later he (1909) corrected the name and discussed at length the two forms which have been recorded by PILSBRY (1901) from Hirado. The conclusion he arrived at was that *Cryptoplax rhodoplax* should be conspecific with *C. japonica*. Since the Japanese species has the tegmenta of various sculptures and the differently coloured articulamenta, both sculpture and colour do not amount to an absolute criterion of the two species.

BERGENHAYN (1933) worked out on the species and pointed out the difference from the results of THIELE especially concerning the structure of radula and stated that the distinguishing character of this species is the median cusp of the major lateral which is exceedingly longer than the outer cusps. But that is not peculiar to the species but the feature characteristic of the genus, and moreover the length of the cusps varies with different position even in one and the same radula. It becomes, therefore, desirable to make a more careful study concerning the structure of tooth in the radula of this species.

### Locality

Sp. No.	STATION	LOCALITY	COLLECTOR	DATE	NUMBER OF SPECIMEN
1744		Kanida	TAKATUKI and SATO	July 23, 1927	8
2045	102	Takaíso, near Sai	HOZAWA, TAKATUKI and SATO	Aug. 17, 1927	1
2054	102	"	"	"	1
2093	104	Ôma	"	Aug. 18, 1927	2
2184	104	"	"	"	1
					Total 13

### Distribution :

#### Pacific coast

Hokkaidô: Hakodate (HILGENDORF).

Honshû: Matusima Bay (TAKEWAKI), Miyagi Pref. Hutomi (HIRASE).

Tiba Pref. Misaki (TAKI, BERGENHAYN); Hayama (TAKI); Enosima (DÖDERLEIN), Kanagawa Pref. Simoda (KANAMARU, KANEKO);

Enoura (HARA); Omaezaki (A. HUZITA), Sizuoka Pref. Wagu (T.



YAMADA, A. HUZITA), Mie Pref. Seto (IW. TAKI); Sirasakimura (K. KANDA); Gobô (OKAMOTO); Tatugahama (KUSE), Wakayama Pref.

Sikoku: Kasiwazima (SUGIMOTO), Kôti Pref. Yawatahama (TAKI), Ehime Pref.

Kyûsyû: Aosima (T. KURODA), Miyazaki Pref. Sakurazima, Kagosima Bay (MITUKURI); Amamiôshima (MITUKURI), Kagosima Pref.

**Inland Sea:**

Honsyû: Awazi (YAGURA), Hyôgo Pref. Onomiti (IW. TAKI), Hiroshima Pref.

Sikoku: Tikami (YAGI); Mitu (TAKI), Ehime Pref.

**West coast:**

Kyûsyû: Amakusa (BABA), Kumamoto Pref. Nagasaki (BUNGE); Hirado (PILSBRY, HIRASE, THIELE); Waniura (HARA); Tusima (MAKI), Nagasaki Pref.

Tyôsen: Kanrin (OKUDA); Zyôzanho (KURIHARA), Saisyûtô.

**Japan Sea:**

Honsyû: Mutsu Bay, Aomori Pref., Toyama Bay (K. KIKUTI), Toyama Pref.

## Family 4 ISCHNOCHITONIDAE

### Genus ISCHNOCHITON GRAY 1847

#### 12. *Ischnochiton comptus* (GOULD, 1859)

Pl. XIV, figs. 5, 10; Pl. XV, figs. 6, 7; Pl. XXV, figs. 9-16; Pl. XXVII, figs. 6, 7; Pl. XXIX, figs. 11-16.

*Chiton (Leptochiton) comptus*

GOULD (1859) Proceedings of the Boston Society of Natural History, Vol. 7, p. 163; Oosima; Bonin and Liu-kiu Is.

— (1862) Otia Conchologica, p. 117.

KURODA (1928) Catalogue of the Shell-bearing Mollusca of Amami-Ôshima, p. 20, no. 213.

*Ischnochiton (Ischnochiton) comptus*

PILSBRY (1893) Manual of Conchology, Vol. 14, p. 117; Philippines (CUMING).

— (1895) Catalogue of the Marine Mollusks of Japan, p. 114.

— (1898) Nautilus, Vol. 12, pp. 50-51.

NIERSTRASZ (1905) Die Chitonen der Siboga-Expedition, S. 24-27, Taf. 1, Fig. 10; Taf. 3, Fig. 77-85; West Lombok; West and North Celebes; Great Sangir Is.; Amboina Is.; Damma Is.; East Sumbawa Is., etc.

THIELE (1910) Zoologica, Bd. 22, Heft 58, S. 111, 113, 114.

KIKUTI, K. (1931) Toyamawan Nantaidôbutu Mokuroku, p. 2, no. 5; Abugasima.

YAGURA (1932) Hyōgokansen Kairui Mokuroku, p. 20, no. 24; Tazima; Harima; Settu; Awazi.

TAKI, IW. (1936) Onomiti Kinkaisan Nantaidōbutu Mokuroku, p. 1, no. 1.

TAKI, IS. (1936) Saitō Hōonkai Hakubukan Zihō, No. 30, p. 1, no. 1; Onahama.

*Ischnochiton thaanumi*

DALL (1926) Proceedings of the Biological Society of Washington, Vol. 39, p. 66; abstract, IS. TAKI (1928) Venus, Vol. 1, no. 1, p. 42; Nago, Okinawa Is. in Loochoo Islands.

KURODA (1928) Catalogue of the Shell-bearing Mollusca of Amami-Ōshima, p. 20, no. 212.

KUSE, Y. (1930) Tatugahama Kinkaisan Kairui Mokuroku, p. 12, no. 182 (part).

*Ischnochiton albrechti*

THIELE (1893) Das Gebiss der Schnecken, S. 382, Taf. 31, Fig. 19; Hakodate (HILGEN-DORF).

*Ischnochiton (Ischnoradsia) hakodadensis*

THIELE (1909) Zoologica, Bd. 22, Heft 56, S. 3.

*Ischnochiton* sp.

TAKI, IS. (1924) Dōbutugaku Zasshi, Vol. 36, No. 429, p. 284, 1 fig.; Misaki.

YOKOYAMA (1931) Catalogue of Marine, Freshwater and Land Shells of Japan, p. 16, no. 461; Iyo.

YAGI, S. (1931) Ehimeken Dōbutu Si, p. 67, no. 735; Tikami.

Chiton of moderate size, shell thin, oval or elliptical in outline, dorsum not prominently elevated, entire surface finely granulated; girdle rather narrow, imbricated regularly with large, solid, smooth, somewhat convexed scales.

Head valve semicircular, with about 40 to 60 divaricate radiating, minutely beaded threads, of which the hindmost very broad, not traversed by the lines of growth, having 11 to 17 slits, usually 11 to 12 in number, arranged rather irregularly around the anterior margin; teeth short, sharp, weakly waved on the outer surface; eaves moderate in width; slit-lines distinct, whitish.

Median valve slightly mucronate, but not keeled; sutural line nearly straight, side slopes roundly arched; jugal area not defined; central areas having the lines of granules either parallel or bending toward the acute jugum, intersected by forwardly diverging series of grooves, forming a zigzag or ripple pattern; granules rather round on the jugal region, becoming elongate oval in shape toward the periphery; lateral areas somewhat elevated, having small radiating furrows, at first 3 to 5 in number, then splitting into 6 to 10, traversed deeply by three or more lines of growth; interior of the valves smooth, not strongly callused, having one slit on each side; sutural laminae moderately extended anteriorly with acute edge; sinus flat, wide; slit-lines rather grooved, provided with very small pores in a row.

**Tail valve:** mucro somewhat elevated, situated slightly in front of the middle; posterior area flat, a little concave, ornamented with about 40 to 50 delicate radial threads; slits 9 to 13, usually 11 in number.

**Girdle:** large scales of perinotum quite smooth, but sometimes marked transversely with numerous, very fine striations, irregularly pitted, usually pectinated at the tip, probably caused by erosion; peripheral scales very small, cylindrical or ovoidal in shape, narrowing slightly toward the tip, smooth on the surface, containing numerous minute granules; large marginal-spines thick, sharply pointed, finely striated; small marginal-spines rod-shaped, smooth with blunt end; marginal spinules claviform with acute tip, weakly striated at the distal end; hyponotum scales hyaline, oblong.

**Colouration:** it is generally light grey in colour with small round white maculations, though variation in colour is great, being (a) densely clouded with olive; (b) light clouded mottled with dark and lilac; (c) four central valves chestnut, the rest olivaceous; (d) brown with olive, with broad white streak down the centre; (e) light reddish-chestnut mottled; (f) the same as e, shading into olivaceous; (g) sixth and eight valves dark olive-brown, the rest light ashy; (h) the whole light ashy; girdle alternately suffused in grey and whitish patches.

**Radula:** central tooth strongly cusped, slightly sinuated at the anterior end, base dilated on both sides; centro-lateral deeply cusped, straight at the front edge, shallowly sinuated at the outer edge, its basal plate nearly triangular in shape; major lateral bicuspidate, inner wing large, a little reflexed at the end, base expanded outwardly so as to form a triangular wing; major uncinus curving inwardly, dilated at the anterior end with a cusp, stalk stout, with a small, squarish base; median marginal bilobed at the posterior end; outer marginal pentagonal shape, directing forward the blunt end.

Gills nearly ambient, ctenidia 22 to 32 in number on each side.

Size: length 15 mm, breadth 10 mm (type).

„ 17.5 mm, „ 10 mm (CUMING)

„ 23 mm, „ 13 mm (pl. XV, fig. 7)

„ 18 mm, „ 11 mm (pl. XV, fig. 6)

„ 16 mm, „ 9.5 mm (pl. XIV, fig. 10)

Divergency: 120° (type), 110° (pl. XXV, fig. 13)

**Remarks:** Rather strange it is that GOULD (1859) placed this species in *Leptochiton*, since the scales are as large, solid and regular as in *Chiton*. Basing on this respect, PILSBRY (1893) treated it as a member of *Ischnochiton* s. str., stating that the median valves have one slit on each side.

As indicated in the diagnosis the insertion plates of this species have generally one slit on both sides, while sometimes two on one side and one on the other and very rarely two on both sides.

The most important distinguishing character of this species is in the girdle scales described by GOULD (1859) thus "the girdle is narrow and imbricated with small elongated grooved scales", and by CARPENTER from CUMING's beautiful specimens, "the girdle has large, solid imbricating scales, sometimes very slightly striated". PILSBRY (1893) remarked in examining the typical specimens, that "the striation of the scales can seldom be seen", and NIERSTRASZ (1905) referred the examples collected in the course of the Siboga-Expedition to Celebes, Lombok and other places, to this species notwithstanding their scales are smooth.

It is difficult to get a clear idea as to the characteristic features of scales whether they are evenly smooth or distinctly striated, as the type was differently described by different authors. NIERSTRASZ is right to refer the material of the Siboga-Expedition to this species, because the valves agree in most respects with those of the type. I think that the original specimens included two forms. Upon examination of numerous specimens collected from the type locality and from other places of Japan, I can clearly distinguish two forms, one of which has smooth and the other distinctly striated scales, both occurring almost always in the same habitat. I do not, therefore, hesitate to consider the specimens of smooth scales as *I. comptus* and that of striated scales as *I. boninensis* of BERGENHAYN (1933).

Since the identification of this form has been very difficult, I asked the late Dr. DALL's assistance in April, 1926 in sending notes and figures and in stating that "the present species seems to ally very closely to *I. comptus* of GOULD, but I can not decide whether or not it is the same species, because the original descriptions too meagre for identification. If this be a new species, I would call it *I. tenuitestus*". Dr. DALL's letter dated June 25, 1926 says that "*I. tenuitestus* is the same as a shell which I named *thaanumi* in a paper appeared in the Proceedings of the Biological Society of Washington (Vol. 39, p. 66) and your specimens differ only in colour". I was, however, not very much satisfied with his letter, because he did not give any notion as to how it should be distinguishable from *I. comptus*. I thought that the form with the striation on the scales was *I. thaanumi* and that with smooth scales *I. comptus*. Recently I have been delighted to have an opportunity of examining the cotype of *I. thaanumi*, kindly sent me by Mr. D. THAANUM of Hawaii. In examining

the cotype it became clear that there are no difference between *I. thaanumi*, and the smooth scaled species which I have hitherto treated as *I. comptus*, in other words, *I. thaanumi* is synonymous with *I. comptus*.

All too fragmentary or insufficient original descriptions often lead the later authors to confusion. For instance THIELE (1893) considered a form of *Ischnochiton* as *albrechti* of SCHRENCK, which was collected by HILGEN-DORF from Hakodate, and afterward he (1909) corrected the name to *I. hakodadensis*, and again he (1910) came to realize that this was neither *albrechti*, *hakodadensis*, *comptus* nor *mitsukurii*, for it had smooth, large, solid scales, though he has not given any further account of it. The radula of this form, figured and described in his work "Das Gebiss der Schnecken", agrees so well that of *I. comptus*, that THIELE's specimens can without a doubt be referred to this species.

### Locality

Sp. No.	STATION	LOCALITY	COLLECTOR	DATE	NUMBER OF SPECIMENS
675	1, (A) (I)	Yunosima	—	—	2
1897	—	"	HOZAWA	Aug. 9, 1927	1
—	—	"	IW. TAKI	Aug. 15, 1930	6
2062	103	Sai Bay	HOZAWA, TAKA-TUKI and SATO	Jul. 17, 1927	1
1716	—	Kanida	TAKATUKI	Jul. 1927	8
—	—	Asamusi	IW. TAKI	Aug. 9, 14, 1930	10
4	—	—	—	—	1
5	—	—	—	—	1
					Total 30

### Distribution

#### Pacific coast

Hokkaidô: Hakodate (THIELE)

Honsyû: Onagawa (TAKI); Matusima Bay (TAKEWAKI), Miyagi Pref. Onahama (TAKI), Hukushima Pref. Kominato; Hutomi, Tiba Pref. (HIRASE). Yokohama (TAKI); Misaki (TAKI, Y. ÔSIMA, TAKEWAKI), Kanagawa Pref. Simoda (KANEKO); Numatu (K. ÔYAMA); Omaezaki (A. HUZITA), Sizuoka Pref. Hatizôzima (UTIYAMA), Izusititô; Titizima (HIROTA), Ogasawara Islands. Seto (IW. TAKI); Gobô

(OKAMOTO); Sirasakimura (K. KANDA), Wakayama Pref.

Sikoku: Naruto (HIROTA), Tokushima Pref. Kasiwazima (SUGIMOTO), Kôti Pref. Yawatahama (TAKI), Ehime Pref.

Kyûsyû: Kagosima (Iw. TAKI); Makurazaki (MITUKURI); Amamiôshima (GOULD, MITUKURI), Kagosima Pref.

Loochoo: Nago, Okinawa Pref. (LANGFORD & THAANUM).

Taiwan: Kiirun (TAKAHASI); Ôtei (S. TAKAHASI).

Philippines (CUMING).

East Indian Islands: Great Sangir Island; Kwandang Bay, North Celebes; Palos Bay, West Celebes; Amboina Is.; Damma Island; East Java; West Lombok Island; Sapeh Bay, Sumbawa Island; South Timor (Siboga-Expedition).

West coast:

Kyûsyû: Kamikosikizima (T. KURODA); Akune (MITUKURI), Kagosima Pref. Amakusa (BABA), Kumamoto Pref. Nisiariie (SIKI); Kisyuku, Gotô (YOSIDA); Tusima (MAKI), Nagasaki Pref.

Tyôsen: Kanrin, Saisyûtô (S. OKUDA); Gunzan, Zenrahokudô (OKUDA).

Inland Sea:

Honsyû: Harima; Settu; Awazi (YAGURA), Hyôgo Pref. Onomiti (Iw. TAKI), Hirosima Pref.

Sikoku: Mitu (TAKI); Tikami (YAGI), Ehime Pref.

Japan Sea:

Hokkaidô: Hukuyama (T. KINOSITA).

Honsyû: Mutsu Bay, Aomori Pref. Kawasaki, Sado (K. KIKUTI), Niigata Pref. Toyama Bay (K. KIKUTI), Toyama Pref. Nanao Bay (T. KURODA), Isikawa Pref. Miyazu Bay (A. IZUKA), Kyôto Pref. Tazima, Hyôgo Pref. (YAGURA). Tazirimura (M. HORI), Tottori Pref. Toriimura (S. TAKAGI), Simane Pref.

Tyôsen: Husan (OKUDA); Ôzyôri (HATAKEDA), Keisyônandô.

### 13. *Ischnochiton paululus*, nov. sp.

Pl. XV, fig. 10; Pl. XXV, figs. 6-8; Pl. XXVI, figs. 6-12; Pl. XXVII, figs. 8, 9.

Body small, 5 mm in length; shell highly elevated with vaulted jugum, punctated all over in quincunx, light reddish purple in colour; girdle narrow, covered with finely striated scales.

Head valve evenly granulated in quincunx, slope steep, straight, smooth; interior of valve smooth, shining, pinkish coloured, not callused, having 10 or 11 slits at the periphery.

Median valve strongly raised at the jugum, but not carinated, side slopes roundly convex, posterior margin slightly beaked; jugum not defined; central area beset evenly with forwardly converging series of elongate, flat, granules, intersected by somewhat stronger forwardly diverging grooves; lateral area slightly elevated, diagonal line inconspicuous, entire surface cut into fine granulation, formed by intersection of two different series of oblique lines of rather rounded granules; interior of valve smooth; callus flat; slit shallow, 1 on each side; slit-line well defined; teeth short, thick, smooth on both surfaces; sutural laminae small, roundly arched at the front edge, widely separated by a flat sinus.

Tail valve very small; sutural line roundly arched; central area much more vaulted than in the median valves; mucro flat, inconspicuous, situated a little behind the middle; diagonal line low, not conspicuous; posterior slope small, steep, deeply concave; sutural laminae small, flat, nearly straight at the anterior edge; slits 9 to 12, arranged rather irregularly at the periphery.

Girdle: perinotum clothed with a little curved, finely grooved scales, grooves usually 15 in number in large scales, peripheral scales very small, strikingly ribbed, its base rhombic in shape; marginal spines long, hyaline, obliquely striated, with small root; marginal spinules very small, hyaline, smooth, pointed at the tip, intermingling sparsely with strongly ribbed, sharply pointed, globular scales; hyponotum scale hyaline, smooth, flat, oblong shaped with somewhat pointed end; peripheral scales bearing a much resemblance to marginal spine, but differing from it by its small size and its deep, longitudinal grooves.

Colouration: shells coloured generally with light reddish purple, pinkish colour predominating in the jugal region except for the median line where it is whitish, painted with dull yellow at the periphery; girdle pale blue, with somewhat regular brownish patches. In another specimen, shell light yellow, maculated with a few, small, brownish freckles; girdle also yellowish, regularly tessellated with dark brown.

Radula: central tooth oblong shaped, having small cusp at the tip, broadly dilate at the base; centro-lateral broad, weakly cusped, sinuated at the outer edge; major lateral bicuspidate, inner wing small, rounded, somewhat thickened at the tip, base exceedingly protruded inwardly; major uncinus long, broad, with weak cusp, its base small, squarish; outer marginal nearly square in shape, slightly protruded at the anterior edge; median marginal bilobed at the posterior end.

Gill: holobranchial, branchiae 13 on each side, extending along the

entire length of foot.

Size and divergency: Body length 5 mm, breadth 3 mm. Divergency 90°.

Remarks: This species resembles Japanese representatives of the genus, *Ischnochiton mitsukurii* and *I. melinus* in its small size, sculpture of valves, striated scales in the girdle and its much elevated shells.

*I. mitsukurii* has the elevated and carinated valves, the three or four radial sulci in the lateral area of the median valves, the straight posterior slope of the tail valve and the coarsely striated scales, whereas in this species the shell is much elevated, but not carinated, and lateral areas are evenly granular and sometimes weakly wrinkled on the surface, the posterior slope is deeply concave and the scales of the girdle are finely grooved.

*I. melinus* has a carinated jugum, evenly granulated lateral areas, a slightly concave posterior area and grooved scales, longitudinally striated marginal spines, not beaked median valves, the lateral areas, however, are not elevated, posterior slope of the tail valve is much broader and less concave and the girdle is more densely clothed with more coarsely striated scales than in this species.

From the young specimens of *I. comptus* it is easily distinguished by the strongly grooved scales and the peculiar features of the marginal armatures and the hyponotum scales. *I. boninensis* differs from the species by having radial sulci in the end valves and the lateral areas, the finely striated large marginal spines, the smooth, long, pointed, small marginal spines, the strongly ribbed marginal spinules and the oblong scales of hyponotum and much more flattened and non-beaked median valves.

Locality: Station 113, off Imabetu, 1 specimen was collected by HÔZAWA on July 21, 1929, sp. no. 2379; 2 small specimens were collected by TAKATUKI together with *Tonicella lineata*, *T. submarmorea*, *Lepidozona mertensi*, *Rhyssoplax kurodai* and *Lepidopleurus assimilis*, off Kanida, in July, 1927, sp. no. 1716.

### Genus ISCHNORADSIA SHUTTLEWORTH 1853

#### 14. *Ischnoradsia hakodadensis* ('CARPENTER' PILSBRY, 1893)

Pl. XV, fig. 8; Pl. XXVI, figs. 1-5; Pl. XXVII, figs. 1-5; Pl. XXVIII, figs. 19, 20.

*Ischnochiton* (*Ischnoradsia*) *hakodadensis*

CARPENTER, MS.

PILSBRY (1893) *Manual of Conchology*, Vol. 14, p. 147, pl. 19, figs. 64-66; Hakodate.

— (1896) *Catalogue of the Marine Mollusks of Japan*, p. 114.



- BERRY (1917) Proceedings of the United States National Museum, Vol. 54, pp. 4-5, pl. 1, figs. 6-7, pl. 3, figs. 3-5, pl. 4, figs. 1-3; Hakodate; Muroran. (Albatross, 1906).  
 KIKUTI (1931) Toyamawan Nantaidôbutu Mokuroku, p. 2, no. 6; Abugasima.  
 TAKI, Is. (1936) Saitôhônkai Hakubutukan Zihô, No. 30, p. 1, no. 3, Aomori Bay; Onahama.

*Ischnochiton hakodadensis*

THIELE (1910) Zoologica, Bd. 22, Heft 56, S. 112, Taf. 8, Fig. 44.

A medium-sized chiton; shell oblong oval in outline, moderately elevated, roundly arched at the back; girdle covered densely with minute scales; shell buff in colour, maculated and striped with dark brown; girdle buff, weakly tessellated with slate blue.

Head valve sculptured with numerous, fine radiating riblets, cut transversely by some concentric growth sulci, having 15-20 slits at the periphery; teeth rather sharp, smooth, though sometimes slightly roughened on the outer surface; eaves short, narrow, solid.

Median valves: lateral area slightly raised, cut into 6 to 9 low, unequal riblets by radiating impressed lines; riblets made uneven by concentric wrinkles of growth; central area very minutely pitted over, formed between quincuncial granulations, arranged in zigzag successions; interior of valve blue-white, having 2-3 slits on each side.

Tail valve: mucro lies a little in front of the middle, rather prominent; posterior slope broad, slightly concave, sculptured radially like the head valve, having 14-15 narrow slits.

Girdle covered with small, solid, convex, very weakly striated, scales; marginal spines yellowish, smooth, rounded at the base with blunt tip, intermingling small marginal spinules, coloured similarly to the spines, strongly ribbed at the tip, with hyaline, oblong plates.

Radula: central tooth oblong, weakly cusped, rounded at the tip, truncated at the base, with square basal plate; centro-lateral weakly cusped, outer wing small, round, basal plate triangular; major lateral bicuspid, inner cusp exceedingly larger, longer than the outer; wing-like expansion very fragile, inserted on the shaft just beneath the cusps with small base; major uncinus long, spoon-shaped, broadly cusped, with small basal plate; outer marginal nearly squarish in outline.

Gills holobranchial, ctenidia 28-32 in number on each side.

Remarks: The specimens exhibit wide range of variability, not only in colour, but in number of ray-ribs on the lateral areas and of marginal slits. The beautiful zigzag sculpture of flattened, overlapping, pointed pustules, which covers the central areas of young specimens, is usually eroded to the pitted appearance characteristic of the adults.

The shell of this species has very much the aspect of *Ischnochiton comptus*, *I. boninensis*, *I. mitsukurii*, *I. zebrinus*, and *I. melinus* from Japanese waters, in the tegmental sculpture, however, from all species of the group *Ischnochiton* this is distinguished by its bicuspidate major lateral, by the presence of the round outer wing of the centrolateral in the radula, by its closely arranged, nearly smooth, convex girdle scales and by the plurality of side slits in the median valves.

*Locality*

Sp. No.	STATION	LOCALITY	COLLECTOR	DATE	No. OF SPECIMEN
1	—	Yunosima	ITO	July 10, 1926	2
2	—	„	HOZAWA	„	1
3	—	„	ITO	„	2
1897	—	„	HOZAWA	Aug. 5, 1927	3
—	—	„	Iw. TAKI	Aug. 15, 1930	20
675	1 (I) A	„	HOZAWA and TAKATUKI	Aug. 5, 1926	1
668	4 (I)	Asamusi	TAKATUKI	May 9, 1926	3
—	—	„	Iw. TAKI	Aug. 9, 1930	20
—	—	„	„	Aug. 14, 1930	15
673	66 (V)	Tozawa	HOZAWA and TAKATUKI	Aug. 11, 1926	1
612	18 (I)	Mourazima	HOZAWA and TAKATUKI	July 17, 1926	1
—	—	„	Iw. TAKI	Aug. 10, 1930	1
1716	—	Kanida	TAKATUKI and SATO	July 23, 1927	20

**Distribution :**

**Pacific coast**

Saghalien : Ōdomari ; Tôbuti (URITA), Aniwa Bay.

Hokkaidô : Akkesi (HADA, OKUDA). Muroran (BERRY) ; Hakodate (PILSBRY, BERRY).

Honsyû : Sakiyamamura ; Miyako ; Hirotamura (TOBA), Iwate Pref.

Onagawa (TAKI), Miyagi Pref. Onahama (TAKI), Hukushima Pref.

**Coast of Japan Sea :**

Saghalien : Maoka (S. MIKI).

Honsyû : Mutsu Bay, Aomori Pref. Toyama Bay (K. KIKUTI).

## Genus LEPIDOZONA PILSBRY 1893

15. *Lepidozona coreanica* (REEVE, 1847)

Pl. XIV, fig. 7; Pl. XXVIII, figs. 1-12; Pl. XXIX, fig. 10; Pl. XXX, figs. 1-5;  
Pl. XXXI, figs. 6, 7.

*Chiton coreanicus*

REEVE (1847) Proceedings of the Zoological Society of London, part 15, p. 24; Korean Archipelago, under stones.

— (1847) Conchologia Iconica, Monograph of the genus Chiton, Vol. 4, pl. 26, fig. 128.

ADAMS, A. and REEVE (1850) The Zoology of the Voyage of H.M.S. Samarang under the command of Captain BELCHER, Mollusca, pl. 15, fig. 9.

*Chiton (Stenosemus) coreanicus*

SCHRENCK (1867) Reisen und Forschungen im Amur-lande, Bd. 2, S. 281-282, Taf. 13, Fig. 1-6; collected by LINDHOLM at the Bay of Hakodate.

*Lophyrus coreanicus*

DUNKER (1882) Index Molluscorum Maris Japonici, p. 157.

*Ischnochiton (Lepidozona) coreanicus*

PILSBRY (1892) Manual of Conchology, Vol. 14, pp. 129-130, pl. 27, fig. 50.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 114.

YAGURA (1916) Hyōgokensan Kairui Mokuroku, p. 1; Awazi, Tazima.

KUSE, Y. (1930) Tatugahama Kinkaisan Kairui Mokuroku, p. 12, no. 183; Tatugahama, Wakayama Prefecture.

YOKOYAMA (1931) Catalogue of Marine, Freshwater and Land Shells of Japan, p. 16, no. 463; Iyo.

*Ischnochiton coreanicus*

HIRASE, Y. (1907) Catalogue of Marine Shells of Japan, p. 21; Teshiwo.

TAKI, IS. (1924) Dōbutsugaku Zasshi, Vol. 36, No. 429, pp. 284-285, with 1 text-figure; Misaki.

BABA (1929) Dōbutsugaku Zasshi, Vol. 41, No. 485, pp. 114-115, text-figs. IV, 5-8, (anatomy).

GISELÉN, T. (1931) Journal of the Faculty of Science Imperial University of Tokyo, Sect. 4, Vol. 2, Part 4, pp. 430, 433, 437, 440, 441; Misaki.

ASANO, H. (1933) Bunrui Suisan Dōbutu Zusetu, p. 523, fig. 352.

*Ischnochiton sp.*

YAGI, S. (1931) Ehimeken Dōbutusi, p. 67, no. 736; Tikami.

*Lorica (Lepidozona) coreanica*

KIKUTI, K. (1931) Toyamawan Nantaidōbutu Mokuroku, p. 2, No. 7; Abugasima.

YAGURA (1932) Hyōgokensan Kairui Mokuroku, (revised edition), p. 20, No. 242; Tazima, Harima, Settu, Awazi.

KURODA, (1935) Miyazakikensan Kairui Mokuroku, p. 39, No. 5; Nangō.

TAKI, IW. (1936) Onomiti Kinkaisan Nantaidōbutu Mokuroku, p. 1, No. 2; Onomiti.

*Lepidozona pectinella*

BERGENHAYN (1933) Kungl. Svenska Vetenskapsakademiens Handlingar, Bd. 72, No. 4, S. 15-19, Taf. 1, Fig. 5; Taf. 2, Fig. 40, 41; Taf. 3, Fig. 42-44, 46; Text-fig. 5; Misaki; Bonin Is.

**TAKI, IS. (1933)** *Venus*, Vol. 4, No. 3, p. 194.

Shell thick, medium sized, oval in outline, rather elevated and keeled at the back, about twice as long as wide; tegmental surface of end valves and lateral areas of median valves radially grooved, central area longitudinally ridged; girdle narrow, tessellated, covered densely with minute scales; colouration brownish or dark greenish, never reddish.

Head valve: semicircular in outline, apex rather low, posterior edge shallowly incised at the middle; ray-ribs low, but prominent toward the margin, provided sparsely with solitary pustules; slits 10 to 17, generally 13 or 14 in number; teeth thick, short, rather acute, but finely roughened inside toward the edge; eaves solid, short; interior of valve smooth, shining, slit-rays indistinct, provided with numerous, small pores.

Median valve square, carinated, scarcely beaked, jugal and pleural areas distinct especially in the 2nd valve; anterior edge nearly straight except for the 2nd valve, elegantly scalloped; lateral area distinctly elevated, sculptured with 3 to 6 ray-ribs, interstices between them slightly concave; tubercles roundly elevated, sparsely spaced; pleural area longitudinally, very finely, granosely ridged, interstices between the ridges deeply hollowed, traversed by somewhat irregular grooves; sutural plate flat, rather truncated at the anterior edge, sinus wide and shallow; slit usually one, rarely two on one side; teeth smooth, short, acute; interior of valve smooth, shining, central region fairly thick at the posterior border; lateral region shallowly concave; slit-ray indistinct, provided with numerous, irregularly arranged pores; anterior edge of the articulamentum between the sutural plates weakly denticulated, usually 6 or 7 in number.

Tail valve: mucro nearly central, low, flat; jugum a little defined from the pleural, both sculptured like that of the median valves, anterior edge slightly arched; posterior area straight, gently sloped, provided with ray-ribs likewise the head valve; slits 12 to 15; teeth thick, short; anterior edge of jugal plate denticulated like that of the median valves; sutural plate flat, wide, truncated at the anterior edge.

Girdle covered densely with small scales which are finely ridged, provided with a small sprout on the front edge; hyponotum covered with oblong, smooth, hyaline scales; margin ornamented with thick, obliquely striated, bluntly pointed spines, long, smooth, sharply pointed spines and small spinules.

Ctenidia holobranchial, along entire length of the foot, with a space between the last gill and the anus.

Colouration: shell generally yellowish brown or dark green, tubercles

yellowish, central areas yellowish, blotched and variegated with dark brown ; in another specimen, whitish or light green, indistinctly mottled and spotted with dark green ; girdle tessellated with dark and pale green ; interior of valves whitish or pale blue with a wide dark green ray on each side near the posterior margin.

Radula : central tooth broad, well arched at the anterior edge, narrowing posteriorly, slightly bilobed at the posterior end ; centro-lateral oblong, winged at the anterior outer corner, basal plate triangular in outline ; major lateral well developed, unequally bicuspidate, outer cusp much weaker than the inner one, its shaft thick, with fairly long appendage at the anterior inner edge : major uncinus simple, broad, weakly cusped ; outer marginal oblong shaped, about twice as long as wide.

Measurements are taken from 86 specimens collected from various localities. The correlation between the body length and the number of gills and the relation between the width of the end valves and the number of rey-ribs are shown in the following figures.

*Graph showing the correlation between the body length and the number of gills*

Body length

	mm	Number of gills																											
		12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60			
	27			1																									
	28			1	1	1																							
	29	1		2																									
	30	1	1		1	2		1																					
	31		1				1	1	1																				
	32						1	1		1																			
	33						1	1	1		4																		
	34						1	1	1	1	1	1																	
	35						1	2	2	1	1	2	4	3	1														
	36							1		2	2	1	2		1														
	37								1	1	2	2	1	1		1													
	38										3	2	2	1		1													
	39												1																
	40											1	1					1		1						1			
	41																	1		1									
	42																									1			
	M	29.5	30.5	28.2	22.9	0.29	3.33	2.33	2.32	5.35	6.34	2.34	7.35	6.37	1.36	5.37	—	37.5	—	40.5	—	40.5	—	—	—	—	41		

The largest example in hand attains 60 mm in length with about 41 gills on each side of the foot. The number of gills increases with the size of body, though it is not in direct proportion to the size ; for example, an individual of 12 mm in length has about 30 gills, while that of 24 mm

about 33, that of 36 mm about 37, and that of 48 mm about 40 on each side. From this it will be seen that when the body becomes twice, three or four times in length, the gills increase in number by 12.5 per cent, 23.3 per cent and 33.3 per cent respectively.

It is the commonest in the littoral zone that individuals about 30 mm long are provided with 34 to 35 gills on each side.

*Graph showing relation between the width of the head and tail valves and the number of their ray-ribs*

		Width of valves														
Number of ray-ribs	mm	4	5	6	7	8	9	10	11	12	13	14	15	16		
	10	II			I											
	12															
	14		1	III	I	I										
	16		1		1 VII	IV	IV	I								
	18			1	I	2 VI	III	IV	I							
	20				3	1	3 IV	IV	V	I						
	22				2	3	4	1	1 IV							
	24					1	3	5	2 I	I						
	26						1	1	3 II	2 III	1					
	28					.		2	4 I	3	2					
	30									3	1		1			
	32									4	2	I	1			
	34									1	1 I					
	36										1					
	38												1			
	40												2			
	42												1	1		

I....V, Tail valve; 1....5, Head valve.

It is obvious from the above table that the largest head valve measures 16 mm in width with 42 ray-ribs and the tail valve 15 mm in width with

Table showing width and number of ray-ribs of head and tail valves

[B] BREADTH OF VALVES (mm)	HEAD VALVE			TAIL VALVE		
	NUMBER OF RAY-RIB [R]	$\frac{R}{B}$	NUMBER OF INDIVIDUALS	NUMBER OF RAY-RIB [r]	$\frac{r}{B}$	NUMBER OF INDIVIDUALS
4				10.0	2.5	2
5	15.0	3.0	2	—	—	—
6	18.0	3.0	1	14.0	2.3	3
7	20.0	2.8	6	15.4	2.2	10
8	21.0	2.6	7	16.9	2.1	11
9	22.3	2.5	11	17.4	1.9	11
10	24.9	2.4	9	18.6	1.8	9
11	26.0	2.3	10	22.1	2.0	14
12	29.8	2.4	13	24.4	2.3	5
13	30.7	2.3	8	34.0	2.6	1
14	—	—	—	32.0	2.2	1
15	38.4	2.5	5	30.0	2.0	1
16	42.0	2.6	1			
		M=2.47	$\Sigma=73$		M=2.06	$\Sigma=68$

M=mean,  $\Sigma$ =totals.

30 ray-ribs. The mode in the table are individuals with head valve of 9 to 12 mm and the tail valve of 7 to 11 mm in width. The number of ray-ribs per one mm in width is 2.47 on an average in the head valve and 2.06 in the tail valve. The head valve is always larger and has more numerous ray-ribs per unit in width than the tail valve.

Remarks: REEVE was the first to describe this species basing upon the material collected from "Korean Archipelago, under stones", and afterwards this has been recorded or collected from various places of Hokkaidô, Honsyû, Sikoku, Kyûsyû, Korea and Formosa. In examining these numerous specimens, it was found that there are some examples which agree precisely with the original description and figure, while most of them differ in sculpture of valve as well as in colouration. Several forms of this group described from the adjacent waters of Japan seem to be identical with this single species. *Lepidozona cultrata* ('CARPENTER'

PILSBRY) (1892), recorded from Hakodate, seems to be a young form of this species. *Lepidozona craticulata* (GOULD) (1859) bears a close resemblance to this species in many respects, differing merely in having more numerous radii of the lateral area. BERGENHAYN (1933) described a new species *L. pectinella* from Misaki which was considered different from *L. cultrata* and *L. craticulata* without any reference to REEVE's works, whereas no difference can not be pointed out from *L. coreanica* in sculpture of the shells, in colouration, in structure of the radula and also in girdle armatures.

### Locality

SP. No.	STATION	LOCALITY	COLLECTOR	DATE	NUMBER OF SPECIMENS
675		Yunosima		Aug. 5, 1926	1
1897		east shore of Yunosima	HOZAWA	Aug. 9, 1927	2
		between Yunosima and Utouzaki	Iw. TAKI	Aug. 15, 1930	5
1568		"	TAKATUKI	June, 11, 1927	1
		north shore of Biol. Labor.	Iw. TAKI	Aug. 14, 1930	3
		Mourazima	"	Aug. 10, 1930	4

### Distribution :

#### Pacific coast

Hokkaidô: Hakodate (1) (SCHRENCK).

Honsyû: Miyako (3), Iwate Pref. Onagawa Bay (4); Matusima Bay (5), Miyagi Pref. Onahama (6), Hukusima Pref. Kominato (7); Hutomi (8), Tiba Pref. Titizima (22), Ogasawara (BERGENHAYN). Yokohama (10); Misaki (9), Kanagawa Pref. Simoda (11); Numazu (12), Sizuoka Pref. Ise Bay (13), Mie Pref. Seto (14); Gobô (15); Tatugahama (16); Wakayama Pref.

Sikoku: Yawatahama (52); Ehime Pref.

Kyûsyû: Nangô (18); Miyazaki Pref. Natui, Birôzima, Sibusi Bay (19), Kagosima (20); Kagosima Pref.

Taiwan (Formosa): Kiirun (21); Kôsyun (53) (TAKAHASI).

#### Inland Sea :

Honsyû: Settu (45); Harima (46); Awazi (50), Hyôgo Pref. (YAGURA). Kozima Bay (47), Okayama Pref. Onomiti (48); Hirosima (49), Hirosima Pref.

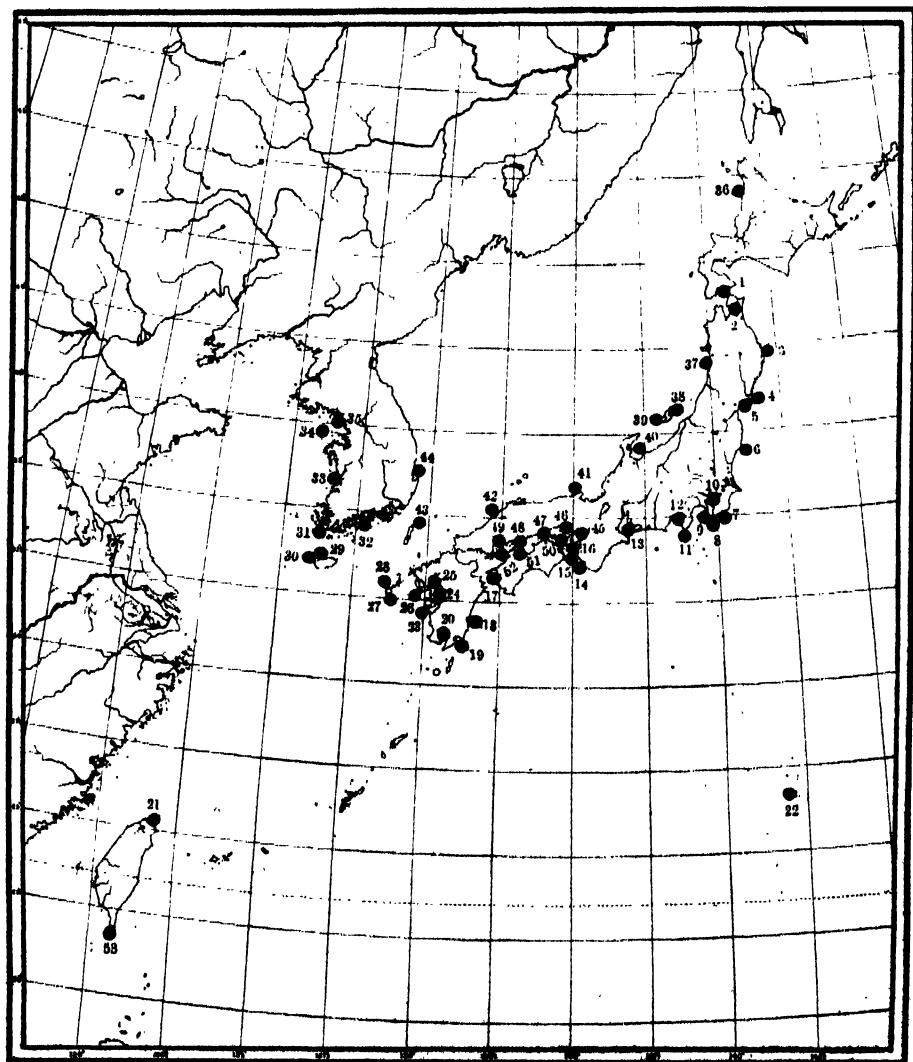


Sikoku : Tikami (51) ; Mitu (52), Ehime Pref.

West coast :

Kyûsyû : Amakusa (23) ; Hyakkanisi (24), Kumamoto Pref. Ariake-kai (25) ; Nagasaki (26) ; Kisyuku (28) ; Tomie (27), Nagasaki Pref.

Tyôsen : Kinnei (29) ; Kanrin (30), Saisyû-tô. Korean Archipelago (31), (BELCHER). Reisui (32), Zenranandô. Gunzan (33), Zenrahokudô.



Text-fig. 2. Geographical distribution of *Lepidosona coreanica* (REESSE).

Tokutumisima (34); Zinsen (35), Keikidô.

Japan Sea coast:

Hokkaidô: Tesio (36).

Honsyû: Mutsu Bay (2), Aomori Pref. Kanaura (37), Akita Pref. Hutami (38); Kawasaki (39), Sado. Toyama Bay (40), Toyama Pref. Tazima (41), Hyôgo Pref. (YAGURA). Torii-mura (42), Simane Pref.

Tusima Straits: Waniura (43), Tusima.

Tyôsen (Korea): Hokô (44), Keisyôhokudô.

# 16. *Lepidozona albrechti* (SCHRENCK, 1863)

Pl. XIV, figs. 8, 9, 14; Pl. XXVIII, figs. 13-18; Pl. XXIX, figs. 7-9, 17, Pl. XXX, figs. 10-13; Pl. XXXI, figs. 1-5; Pl. XXXII, fig. 17.

## *Chiton albrechtii*

SCHRENCK (1863) Bulletin de l'Académie Impériale des Sciences de St.-Petersbourg, Tome 5, p. 551.

— (1863) Mélanges biologiques tirés de Bulletin de l'Académie Impériale des Sciences de St.-Petersbourg, Tome 4, p. 253.

— (1867) Reisen und Forschungen im Amur-Lande, Bd. 2, S. 283-288, Tab. 13, fig. 7-17; Hakodate.

## *Chiton (Lepidopleurus) albrechti*

SMITH, E. A. (1875) Annals and Magazine of Natural History, Ser. 4, Vol. 16, p. 715; Endermo Harbour.

## *Ischnochiton (Ischnoradsia) albrechti*

PILSBRY (1893) Manual of Conchology, Vol. 14, pp. 147-148, pl. 19, figs. 70-74; Hakodate.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 114.

BERRY (1917) Proceedings of the United States National Museum, Vol. 54, no. 2223, p. 4; pl. 1, figs. 4-5; pl. 3, figs. 1-2; text-fig. 1; Muroran.

NIERSTRASZ (1905) Die Chitonen der Siboga-Expedition, Monographie no. 41, S. 22.

KINOSHITA, T. and ISAHAYA, T. (1934) Hokkaidôsan Kairui Mokuroku, p. 3, no. 1, pl. 1, fig. 1; Okusiri, Yoiti, Risiri.

## *Lorica (Lepidosona) albrechti*

KIKUTI (1931) Toyamawan Nantaidôbutu Mokuroku, p. 2, no. 8; Takosima, Noto.

## *Lepidozona albrechti*

TAKI, IS. (1936) Saitô Hôonkai Hakubutukan Zihô, No. 13, no. 5; Kamomezima, Aomori-Bay; Onahama, Hukushima Prefecture.

Body large, oval, elevated with acute dorsal ridge; entire surface of shells minutely punctated, reddish in colour, maculated with dark brown; girdle narrow, covered densely with imbricating scales.

Head valve having rather fine, low ray-ribs, bearing sparsely brown pustules; majority of ribs splitting into two toward the margin; teeth

short, acute, roughened on the outer surface; slits 14 or more in number.

Median valve square with nearly straight side slopes; jugum not defined except for the 2nd valve; central area with about 30 longitudinal lines of granules, in juvenile shells the area sculptured with longitudinal and transverse lines so as to form a fine reticulation, nodes of which remain in the adult shells as granules in a longitudinal series; lateral area scarcely elevated, having 4 to 6 in the juvenile, 10 to 12 radiating, sparsely granose riblets in the adult; in old shells entire surface non-granulated and distantly concentrically sulcated; sutural laminae short with regularly arched at the anterior edge; sinus very flat with narrow, denticulated lamina.

Tail valve smaller than head valve; mucro median, nearly flat; posterior area straight, gently sloped, ornamented with ray-ribs like the head valve; sutural laminae narrow, roundly arched; sinus flatter than in the median valves; interior of valve white, tinted with rays of reddish-brown; teeth thick, acute, sometimes rugose or lobed on the outer surface; slits deep, 12 in number.

Girdle moderately wide, reddish-brown or brownish-yellow, tessellated regularly with dark-brown, covered with convex scales which are weakly striated, usually 10 in number, bearing a small process at the front end, sinuated at the middle of the base; margin of girdle armed with long, smooth, acute bristles, striated thick spines and small striated acute spinules; hyponotum covered densely with hyaline, more or less elongated cylindrical scales.

Radula: Central tooth dilated and weakly undulated at the anterior edge, constricted at a trifle behind the middle with a little broadened base; centro-lateral square with a large appendage at the anterior outer corner; major lateral bicuspidate, cusp distinct in juvenile specimens, being stronger in the inner one, in adult specimen it looks sometimes like unicuspid the smaller one having worn out; stalk thick with a long appendage at the anterior end of the inner edge and a small process at the outer edge and dorsal processes; major uncinus long, stout, slightly cusped at the anterior edge; outer marginal oblong, being longer than wide.

Gills holobranchial with a small space between the last gill and the anus.

Remarks: PILSBRY (1893) treated this species as a member of the subgenus *Ischnoradsia* under the genus *Ischnochiton* and this was followed by BARRY (1917). As pointed out by SCHRENCK (1863) this species is closely related to *Lepidozona coreanica* and the features characteristic of

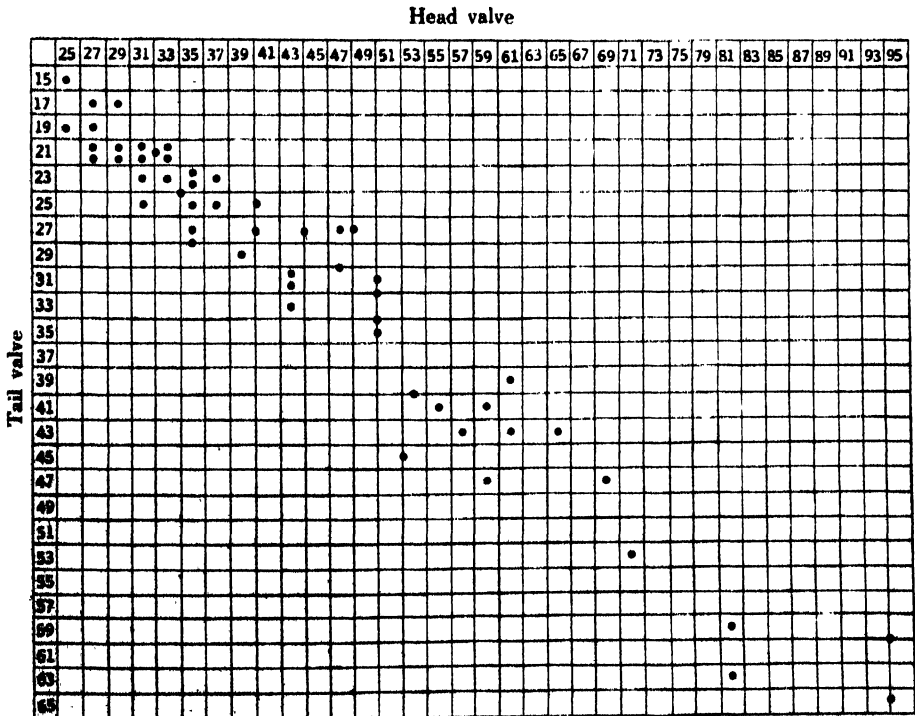
this species seem to agree with that of *Lepidozonia*. It is, therefore, more natural to consider this species as a member of *Lepidozonia* than as that of *Ischnoradsia*.

SMITH (1875) reported two large specimens from Muroran (Endermo Harbour), which measured about 65 mm in length and 28 mm in breadth in the central valves. These specimens are the largest, because it usually measures from 40 to 50 mm in length.

The ratios of the length to the width range from 1.56 to 1.83 and the mean ratio is 1.73 as shown in the following table:

Table showing length and width of body, and ratios of length to width

LENGTH	WIDTH	WIDTH LENGTH
22 mm	14 mm	1.56
33	20	1.65
44	26	1.69
42	24	1.76
46	26	1.76
46	26	1.76
50	28	1.78
48	27	1.77
49	28	1.75
55	30	1.83
		M=1.73



Text-fig. 3. Graph to show relation between head and tail valves respecting the number of ray-ribs.

In the above table it will be seen that the ratios increase with the length of the body and that the body becomes relatively narrower as it grows older.

The number of ray-ribs on the end valves increases regularly in proportion to the body size and is always more numerous in the head valve than in the tail one and its relation between both valves is plotted in text-fig. 3.

The number of slits ranges from 11 to 17 in the head valve and from 10 to 16 in the tail valve and is always more numerous in the former than the latter. Both valves have each about 14 and about 12 slits on an average. The following table shows the relation between the number of slits in the end valves.

NUMBER OF SLITS				
	HEAD VALVE		TAIL VALVE	
I	II	I × II	II	I × II
10			4	40
11	1	11	5	55
12	4	48	5	60
13	3	39	3	39
14	3	42	0	0
15	2	30	1	15
16	1	16	1	16
17	4	68		
Σ	18	254	19	225
M		14.1		11.8

I, number of slits; II, number of individuals; M, mean; Σ, totals.

SCHRENCK gives 112° in the divergency of the median valve. So far as the materials examined it varies from 100° to 123° and the mean is about 108°

The ctenidia extend along the practically entire length of the foot and the number of gills ranges from 25 to 50, being equal or nearly so on

DIVERGENCY	F	DIVERGENCY	F
100°	3	112°	2
102	2	113	1
105	1	114	1
106	2	115	1
108	1	117	1
110	3	123	1
		$\Sigma = 2065$	$= 19$
		$M = 108.63$	

Table showing variation of divergency in median valves.

F, number of individuals;  $\Sigma$ , totals; M, mean.

Body length

mm	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56
37	1																	
38	1																	
39																		
40																		
41																		
42							1				1							
43													1					
44							1											
45											1	2		1				
46											1			1				
47												1	1		1			
48														1	2			1
49																		
50																		1

Text-fig. 4. Graph showing the relation between the body length and the number of gills.

each side and increases in proportion to the length of body as shown in the following table and graph.

NUMBER OF GILLS		F
LEFT	RIGHT	
26	26	1
28	28	1
29	29	1
25	30	1
30	30	3
32	30	1
31	31	2
31	32	1
32	33	1
33	33	1
35	37	1
37	38	1
40	40	1
41	41	1
44	42	1
45	43	1
45	47	1
45	48	1
46	42	1
47	45	1
48	46	1
47	48	1
48	50	1
Σ		26

BODY LENGTH	NUMBER OF GILLS	
	LEFT	RIGHT
22 mm	37	38
33	44	42
42	46	42
44	45	47
46	45	43
46	47	45
48	48	46
49	47	48
50	45	48
55	48	50

Table showing the relation between body length and number of gills on both sides.

F, number of individuals; Σ, totals.

*Locality :*

Sp. No.	STATION	LOCALITY	COLLECTOR	DATE	NUMBER OF SPECIMENS
670	1 (I)	Ôsima, Asamusi	HOZAWA and ITO	July 12, 1926	1
672	83 (III)	Akimae	KOKUBO and KAMADA	Aug. 23, 1926	2
674	31 (I)	Itasaki	HATAI and HOZAWA	July 24, 1926	2
676	15 (I)	Ôsima	HOZAWA and TAKATUKI	July 16, 1926	1
678	76 (III)	Akimae	KOKUBO and KAMADA	Aug. 22, 1926	1
679	63 (V)	Syukunobe			5
681	21 (I)	Hanakuri, Nonai-mura	HOZAWA and TAKATUKI	July 19, 1926	2
683	—	—	—	—	3
686	23 (I)	Moura	HOZAWA and TAKATUKI	July 20, 1926	2
1639	—	Kamomezima	TAKATUKI	July 10, 1926	1
1722	—	Kanida	TAKATUKI and SATO	July 23, 1927	1
1897	—	Yunosima	HOZAWA	Aug. 9, 1927	1
—	—	„	Iw. TAKI	Aug. 15, 1930	10
—	—	Asamusi	„	Aug. 14, 1930	1
—	—	„	„	Aug. 9, 1930	1

**Distribution :****Pacific coast**

Hokkaidô: Akkesi (1) (HADA and OKUDA); Muroran (2) (SMITH, BERRY); Hakodate (3) (SCHRENCK, PILSBRY, K. ENDÔ).

Honsyû: Miyako (5); Kesenuma (6), Iwate Pref. Onagawa (7), Miyagi Pref. Onahama (8), Hukusima Pref.

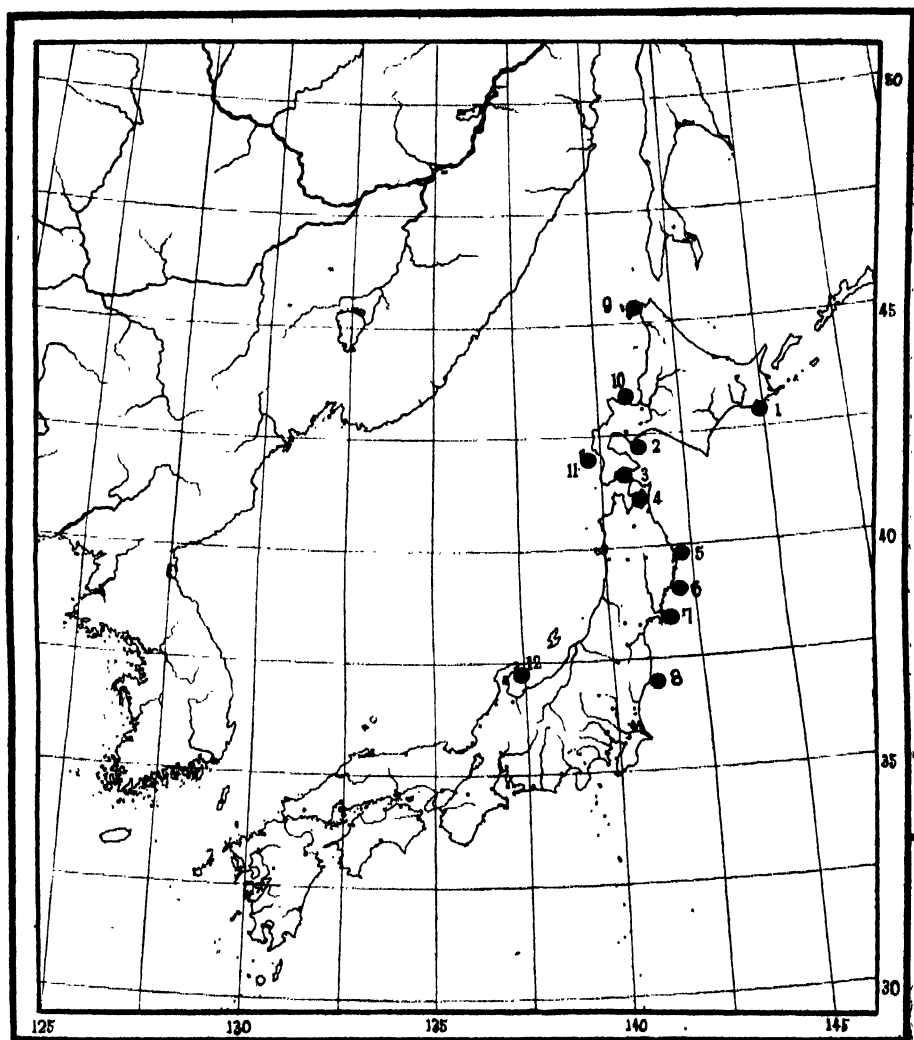
**Coast of Japan Sea**

Hokkaidô: Risiri-tô (9); Yoiti (10); Okusiri-zima (11) (KINOSITA and ISAHAYA).

Honsyû: Mutsu Bay (4), Aomori Pref. Toyama Bay (12) (KIKUTI), Toyama Pref.

This is one of the commonest species at the littoral zone of Hokkaidô and Honsyû with the southern limit in its distribution at about 37° N on both sides of Honsyû.





Text-fig. 5. Geographical distribution of *Lepidozona albrechti* SCHRENCK.

### 17. *Lepidozona mertensi* (MIDDENDORFF, 1846)

Pl. XIV, fig. 6; Pl. XXIX, figs. 1-6; Pl. XXX, figs. 6-9; Pl. XXXI, figs. 9, 10.

#### *Chiton mertensii*

MIDDENDORFF (1846) Bulletin de la Cl. Phys.-mathem. de l'Académie de St.-Pétersbourg.  
Tome 6, p. 118.

#### *Chiton (Stenosemus) mertensii*

MIDDENDORFF (1847) Melacologia Rossica, pp. 34, 125-127, Tab. 14, Fig. 1-3; (1848)

Mémoires de l'Académie Impériale de Sciences de Saint-Petersbourg, Ser. 6, Tome 6, pp. 189-191; California, coll. by ROSS. MERTENS.

*Leptochiton mertensii*

ADAMS, A. and H. ADAMS (1854) Genera of Recent Mollusca, Vol. 1, p. 473.

DUNKER (1882) Index Molluscorum Maris Japonici, p. 158; Hakodate.

*Lepidopleurus mertensii*

DALL (1878) Proceedings of the United States National Museum, Vol. 1, pp. 297-332.

— (1878) Bulletin of the United States National Museum, Vol. 1, pp. 79, 111-115, pl. 2, figs. 18, 18 a. (radula), Sitka, south to Monterey.

*Ischnochiton (Lepidozona) mertensii*

PILSBRY (1892) Manual of Conchology, Vol. 14, pp. 125-126, pl. 26, figs. 20-26.

— (1895) Catalogue of the Marine Mollusks of Japan, p. 114.

BERRY (1917) Proceedings of the Californian Academy of Sciences, Ser. 4, Vol. 7, No. 10, p. 236; Forrester Island; Waterfall Cannery, Prince of Wales Island, Alaska.

DALL (1921) Smithsonian Institution United States National Museum, Bulletin 112, p. 192; Sitka, Alaska, to San Pedro, San Martin Island, Lower California, cf. Addit. and emend. etc., Proc. U. S. Nat. Mus., Vol. 63, pp. 1-4.

BERRY (1922) Proceedings of the Californian Academy of Sciences, Ser. 4, Vol. 11, No. 18, pp. 475-476, pl. 10, figs. 7-12.

— (1926) American Journal of Science, Vol. 12, p. 456; Pleistocene of San Quintin Bay, Lower California.

— (1927) Proceedings of the Malacological Society of London, Vol. 17, p. 164; China Hat, Queen Charlotte Islands, British Columbia.

*Ischnochiton mertensi*

CHACE (1930) Nautilus, Vol. 44, pp. 7-8.

Shell oval, moderately thin, elevated with angular dorsal ridge; terminal and lateral areas radially pustulose; central area strongly lired; girdle narrow, covered densely with small scales.

Head valve ornamented with 25 to 35 ray-ribs, each bearing a series of rounded or pyriform pustules, the posterior series directed obliquely backward so as to dentate the suture; apex low, inconspicuous, interior of valve smooth; slit-ray distinct, without a pore; slits 9 to 11.

Median valve carinated at the jugum with straight side slope; lateral area distinctly raised, sculptured with radiating series of pustules, 5 or 6 in row; central area having acute, narrow, longitudinal riblets, interspace between them roughly latticed across, obsolete at the dorsal ridge, where the riblets have a tendency to diverge; jugal area distinct only in the 2nd valve, provided with diverging lirae with nearly smooth interspaces; sutural laminae low, wide, roundly arched at the edge, connected across the sinus by a dentate plate; teeth short, obtuse, usually roughened on the outer surface; eaves solid, wide; one slit on each side; sinus flat, angular at the base; interior of valve smooth; callus indistinct; slit-ray provided with numerous, fine, elongate pores.

Posterior area of tail valve straight, radially pustulose as in the head valve, central area roundly arched, sculptured with 20 to 24 sharply cut, longitudinal bars, traversed by numerous irregular grooves; mucro median, low, inconspicuous; slits 10 to 12.

Girdle firm, compactly covered with regular, solid scales, which are nearly squarish in outline, fairly curved inward, ornamented with about 10 longitudinal ribs, having a process at the front end; marginal armatures composed of three kinds of spines, the longest of which is hyaline, slightly curved, cylindrical of same diameter, sparsely beset among the thick, short, hyaline, striated spines; the smallest brownish in colour, sharply pointed at the end, strikingly striated near the tip; hyponotum covered densely with hyaline, oblong scales.

Colouration: Varying in colour from orange-red to claret-red, or even dark red-brown, either unicoloured or speckled and blotched with white; interior of valves generally white or blue-white; median valves showing broad red-brown rays posteriorly, tail valve tinted with crescents of the same colour at the middle; colouration of girdle similar to that of the shells, regularly or obscurely tessellated in two shades of dark brown.

Radula: Central tooth dilated anteriorly, slightly sinuated at the front edge, shallowly constricted on both sides; centro-lateral squarish in outline, having a large outer wing at the anterior end, a broad outer lamella at the posterior end; anterior edge slightly sinuated at the middle, though not cusped; major lateral bicuspidate, outer cusp much smaller than the inner one; outer process of the shaft remarkably protruded outside at about the middle, dorsal surface deeply channelled; major uncinus slightly curved inward with large basal plate; marginals oblong shaped, the outer one narrower anteriorly.

Gill rows take the space three-quarters of the foot, each containing about 40 branchiae.

Size: Length 20 mm, breadth 6 mm; divergency 100° (DALL)

„ 18 mm, „ 12 mm; „ 105°

„ 17 mm. „ 11 mm; (contracted specimens).

Locality: Kanida, 5 specimens were collected by TAKATUKI in July, 1927; sp. no. 1716.

Distribution: On the Pacific coast of America, Sitka and vicinity, south to Lower California, on the Japanese coast, Hokkaidô and northern Honsyû.

#### American coast

Alaska; Forrester Island; Waterfall Cannery, Prince of Wales Island

(BERRY); Sitka (DALL).

British Columbia; China Hat, Queen Charlotte Islands (BERRY); Puget Sound Region (BERRY); Northern California (BERRY); Monterey Region (DALL, BERRY); Southern California (BERRY); San Pedro, San Martin Island (DALL, BAKER); San Quintin Bay (BERRY), Lower California (BERRY).

Japanese coast

Hokkaidô; Hakodate (DUNKER).

Honsyû; Mutsu Bay.

## Family 5. CHITONIDAE

### Subfamily A. CHITONINAE

Genus RHYSSOPLAX THIELE 1893

#### 18. *Rhyssoplax kurodai* (Is. TAKI et Iw. TAKI, 1929)

Pl. XV, fig. 5; Pl. XXXI, fig. 11; Pl. XXXII, figs. 1-7.

*Chiton* sp.

TAKI, Is. (1924) *Dôbutugaku Zasshi*, Vol. 36, pp. 285-286, with 1 text-fig.; Misaki, Sagami.

YAGI (1931) *Ehimeken Dôbutusi*, p. 67, no. 737; Tikami.

*Chiton kurodai*

TAKI, Is. and Iw. TAKI (1929) *Venus*, Vol. 1, No. 2, pp. 52-53, pl. 2, fig. 3, text figs. 8-11; Iyo; Misaki; Seto.

KUSE (1930) *Tatugahama Kinkaisan Kairui Mokuroku*, p. 12, no. 185; Tatugahama, Wakayama Pref.

KIKUTI (1931) *Toyamawan Nantaidôbutu Mokuroku*, p. 2, no. 9; Abugasima, Toyama Bay.

YOKOYAMA (1931) *Catalogue of Marine, Freshwater and Land Shells of Japan*, p. 16, no. 468; Iyo.

YAGURA (1932) *Hyôgokensan Kairui Mokuroku*, p. 20, no. 246; Harima; Awazi.

*Chiton (Rhyssoplax) kurodai*

TAKI, Iw. (1936) *Onomiti Kinkaisan Nantaidôbutu Mokuroku*, p. 1, no. 5, Onomiti.

TAKI, Is. (1936) *Saitôhônokai Hakubutukan Zihô*, No. 30, p. 1, no. 13; Onahama, Hukushima Pref.

*Chiton (Clathropleura) bocki*

BERGENHAYN (1933) *Kungl. Svenska Vetenskapsakademiens Handlingar*, Bd. 12, No. 4, S. 28-29, Taf. 1, Fig. 8, Taf. 3, Fig. 53, Text-fig. 9, a-f; Sagami Misaki.

Body oval or elongate oval in outline; shells well elevated, weakly carinated at the jugum, moderately beaked on the median valves; girdle rather narrow, densely covered with imbricating scales. It is one of the handsomest chitons in Japan.

Head valve thick, solid, generally smooth on the tegmental surface, it

is finely granulated in quinquex when seen under a microscope; anterior edge regularly arched; posterior edge straight, shallowly incised at the apex; interior of the valve smooth, shining, without a distinct callus; slits deeply incised, 8 to 9 in number; slit-lines shallow, distinct, provided with numerous small pores.

Median valves oblong shaped with an arched front edge and a well protruded beak; lateral area distinctly raised, smooth like the head valve; jugum not distinct, only defined by its smoothness from the pleural area, which is sculptured with 14 to 21 longitudinal lirae; sinus wide, flat, with pectinated laminae at the base; interior of valves polished with well developed callus at the middle; lateral region deeply concave; jugal region provided with numerous transverse, short lines; sutural laminae low, roundly arched, sharp at the front edge; teeth thick, solid, strikingly pectinated on the outer surface; slit distinct, one on each side; slit-line wide, provided with pores in double rows; posterior edge broadly reflexed.

Tail valve semicircular in outline, nearly straight at the front edge, regularly round posteriorly; mucro a little in front of the middle, distinct, though not raised; posterior slope slightly concave, sharply demarcated with diagonal lines from the central area, which is sculptured with 11 to 14 longitudinal lirae; jugum inconspicuous; interior of the valve smooth, without any callus; teeth thick, solid, well pectinated as in those of the median valves; slit-lines like those of the head valve; slits distinct, usually 9 to 12 in number; sutural laminae flat, wide, truncated at the front edge.

Perinotum of the girdle covered densely with smooth, regularly imbricating, strongly curved scales with rhombic base, generally from 16 to 18 in a diagonal row, tending to become smaller toward the edge; marginal spinules small, hyaline, lanceolate with somewhat flattened base; hyponotum covered with minute hyaline, oblong scales.

Teeth of the radula weak except for the major laterals; central tooth of much elongate oblong shape with a long, tapering cusp at the tip; centro-lateral rhombic shaped, with a remarkable folds in front, nearly straight on both inner and outer edges, slightly waved at the posterior edge, having a small appendage near the tip on the outer edge, its basal plate triangular, very much thickened at the inner edge; major lateral thick, stout, having a narrow outer wing and a small, oblong inner process, concave at the back near the tip, holding a large, entire, obtusely pointed cusp at the anterior end; major uncinus very small, broadly dilated at the tip so as to take the shape of a fan, stalk short, basal-plate small,

oval shaped; inner marginal small, oblong with inconspicuous central process, waved at the outer edge; middle-marginal larger than the former, elongate hexagonal shaped; outer marginal squarish in outline, slightly protruded at the posterior edge.

Ctenidia holobranchial abanal, gills about 27 on one side.

Remarks: *Chiton bocki* was described by BERGENHAYN (1933) basing on the material collected by SIXTEN BOCK at Misaki and was considered different from *Chiton canariensis* and *Ch. rhynchotus*, recorded from the Canary Islands and New Caledonia respectively, in having the smooth scales in the girdle and the denticulated jugal plate. No reference was made by him in his paper to the known Japanese species of the genus e. g., *Chiton aquatilis*, *Ch. densiliratus* and *Ch. kurodai*. The last species was reported by myself (1924) from Misaki, named by my brother and me (1929) basing on the material from Misaki, Seto and Mitu, and does not show any difference from *Ch. bocki* in all important features of the valves, the scales, the radula, the ctenidia and the colouration.

Locality: Kanida, two small specimens were collected by TAKATUKI in July, 1927 together with *Tonicella lineata*, *Tonicella submarmorea*, *Lepidozona mertensi*, *Lepidopleurus hakodatensis* and *Ischnoradsia hakodadensis*; sp. no. 1716. 15 specimens were collected by IWAOKI TAKI at Yunosima on August 13, 1930.

Distribution:

Pacific coast

Honsyû: Matusima Bay, Miyagi Pref. (TAKEWAKI). Onahama, Hukushima Pref. (TAKI). Misaki, Kanagawa Pref. (TAKI, BERGENHAYN). Kamizusima, Izusitôtô (MIYAZI). Simoda, Sizuoka Pref. (KANEKO). Seto (TAKI), Tatugahama (KUSE), Wakayama Pref.

Sikoku: Kasiwazima, Kôti Pref. (SUGIMOTO). Yawatahama, Ehime Pref. (TAKI).

Inland Sea

Honsyû: Harima; Awaji, Hyôgo Pref. (YAGURA). Onomichi, Hiroshima Pref. (Iw. TAKI).

Sikoku: Imaharu (TAKI); Tikami (YAGI); Mitu (TAKI), Ehime Pref.

West coast

Kyûsyû: Simokosikizima, Kagosima Pref. (MITUKURI).

Tyôsen: Kanrin, Saisyûtô (OKUDA).

Japan Sea

Hokkaidô: Hukuyama, Ozima (KINOSITA).

Honsyû: Mutsu Bay, Aomori Pref. Tobisima, Akita Pref. (KURODA).

Abugasima, Toyama Bay (KIKUTI). Toriimura, Simane Pref. (S. TAKAGI).

19. *Rhyssoplax tectiformis*, nov. sp.

Pl. XV, fig. 4; Pl. XXXI, fig. 8; Pl. XXXII, figs. 8-13.

Body of small size; shell ovate in outline, strongly elevated at the jugum, central area weakly lired, other areas smooth; mucro subcentral; girdle covered with small scales, orange red in colour, tessellated with brownish red.

Head valve: apex bluntly prominent, posterior margin incised at the middle; surface generally smooth, but showing a very minute quincuncial pattern of granulation under the lens, marked faintly with concentric lines, maculated with reddish brown; interior of valve smooth, reddish brown in the middle, brownish yellow at the periphery; teeth thick, short, well pectinated on the outer surface, eaves solid, narrow; slits shallow, 9 in number.

Median valves strongly keeled at the jugum, much beaked at the posterior margin; front edge slightly sinuated at the middle; jugal area not well defined; whole surface minutely punctated like the head valve; central area ornamented with about 10 weak longitudinal lirae on each side of the keel, maculated irregularly with brownish red; lateral area very much raised, marked with a few concentric lines of growth; interior of the valves light pinkish in the pleural region, chestnut in the jugal region, brownish red in the lateral region; callus not thickened; slit-line shallowly grooved; teeth thick, short, well pectinated on the outer surface; slit one on each side; sutural laminae thin, short, rounded at the front edge; sinus wide, shallow, with small plate at the base, composed of five, small, wedge-shaped plates.

Tail valve: mucro prominent, situated at about two-fifths the entire length from the anterior edge; central area having 6 lirae on each side, separated by low ridges from the posterior area, which is slightly concave, sculptured like the head valve; sutural laminae short, wide, truncated at the front edge; interior smooth, brownish red at the middle, brownish yellow at the posterior margin, whitish anteriorly; slits at the periphery 11 in number.

Girdle: scales of perinotum squarish in outline, smaller ones more rounded with broad, rhombic base; surface smooth, though in large ones marked with fine, transverse lines; marginal spines small, hyaline, striated

obliquely, pointed bluntly at the tip, truncated at the base; scales of hyponotum hyaline, flattened rod-shape, blunt at the anterior end.

Radula: central tooth much elongated, strongly cusped at the front end, truncated at the base; centro-lateral broad, much undulated at the front edge, outer lamella protruded anteriorly, bearing a small wing at the outer surface near the tip; major lateral well developed, shaft stout, inner wing large, slightly reflexed at the end, cusp thick, simple, not acute at the edge; major uncinus small, fan-shaped, stalk thick, short with small basal plate; outer marginal much wider than long.

Gills: holobranchial, extending along the entire length of foot, 23 or 24 ctenidia on each side.

Size: body length 9 mm, breadth 6 mm (type).

„ „ 10 mm, „ 6 mm (cotype).

Head valve 3.25 mm, 2nd valve 3.7 mm, 3rd valve 4 mm, tail valve 2.8 mm in breadth (type).

Colouration: Orange red on the whole surface, the shells maculated with brownish red; in the cotype the second valve and the central area of the third valve are brownish yellow; the girdle finely tessellated.

Remarks: This species bears a much resemblance to *Chiton kurodai* in the texture of the shells, the sculpture of the central area, the shape of the scale in the girdle and of cusps in the radular teeth. This differs from it in having much coarser texture of shells, much less number of lirae in the central area, much more elevated and beaked valves, the smaller, striated marginal spines, the smaller cusp of the central tooth, much more developed inner wing of the major lateral, the transversely

CHARACTERS	SPECIES	<i>tectiformis</i>	<i>kurodai</i>
	number	9-10	12-13
lirae	shape	ridge	groove
beak		more beaked	less beaked
laminae of sinus		cut into five plates	denticulated
central tooth		cusp simple, small	tapering, long cusp
major lateral		wing large, cusped	wing small, not cusped
outer marginal		oblong	squarish
marginal spine		obliquely striated, small, hyaline, obtuse	smooth, large, brown, sharply pointed



elongate outer marginal. These differences are shown in the above table.

Locality: Ōma, 2 specimens were collected by HÔZAWA, TAKATUKI and SATÔ in August, 1927; station 104; specimen number 2076, 2077.

## Subfamily B. ACANTHOPLEURINAE

### Genus LIOLOPHURA PILSBRY 1893

#### 20. *Liolophura japonica* (LISCHKE, 1873)

Pl. XV, fig. 3; Pl. XXXII, figs. 15, 16; Pl. XXXIII, figs. 1-8; Pl. XXXIV, figs. 1-4.

##### *Chiton spiniger*

SCHRENCK (1867) Reisen und Forschungen im Amur-Lande, Bd. 2, S. 275-276; Hakodate.

##### ? *Chiton de-Filippii*

TAPPARONE-CANEFRI (1874) Zoologia del Viaggio Intorno al Globo della Regia Fregata Magenta, p. 77; Japan, not *Amycula de-filippii*.

##### *Chiton japonicus*

LISCHKE (1873) Malakozologische Blätter, Bd. 21, S. 22.

— (1874) Japanische Meeres-Conchylien, Theil 3, S. 71, Taf. 5, Fig. 8-11; Nagasaki.

##### *Chaetopleura japonica*

DUNKER (1882) Index Molluscorum Maris Japonici, p. 158; Nagasaki.

##### *Acanthopleura japonica*

THIELE (1893) Gebiss der Schnecken, Bd. 2, Lfg. 8, S. 373, Taf. 30, Fig. 34; Enosima (DOEDERLEIN).

##### *Acanthopleura (Liolophura) japonica*

THIELE (1910) Zoologica, Bd. 22, Ht. 56, S. 3, 115.

NOWIKOFF (1907) Zeitschrift für wissenschaftliche Zoologie, Bd. 88, S. 153-186, Taf. 10, Fig. 2-4, 9, Taf. 11, Fig. 14.

##### *Liolophura japonica*

PILSBRY (1893) Manual of Conchology, Vol. 14, pp. 242-243, pl. 53, figs. 41-44; Enosima (STEARNS).

— (1895) Catalogue of the Marine Mollusks of Japan, p. 115, Enosima (STEARNS).

NIERSTRASZ (1905) Notes from the Leyden Museum, Vol. 25, p. 115, pl. 10, fig. 22; Japan.

— (1905) Siboga-Expedition, Monographie 48, p. 108; Japan, Enosima, Nagasaki.

HIRASE, Y. (1907) Catalogue of Marine Shells of Japan, p. 23; Hirado, Hizen

— (1909) Kairui Tebikigusa, p. 69, fig. 68.

— (1910) Nippon Senkai Mokuroku, p. 30, no. 633; Hizen.

— (1914) Hutû Kairui no Siori, p. 1.

— (1915) Conchological Magazine, Vol. 4, no. 1, pl. 1, fig. 8.

YAGURA, W. (1913) Nippon Kairui Syasin Tyô, pl. 44.

— (1916) Hyôgoken Kairui Mokuroku, p. 2, no. 5; Awazi; Settu; Harima; Tazima.

TAKI, Is. (1924) Dôbutugaku Zasshi, Vol. 36, No. 429, pp. 289-290, 1 text-fig.; Misaki, Sagami.

HIRASE, S. (1927) Figuraro de Japanaj Bestoj, p. 1503, fig. 2888.

- BABA, K. (1929) *Dōbutugaku Zassi*, Vol. 41, No. 485, pp. 108, 115-116, text-figs. IV, figs. 1-4.
- THIELE (1929) *Handbuch der systematischen Weichtierkunde*, S. 21.
- KUSE, Y. (1930) *Tatugahama Kinkaisan Kairui Mokuroku*, p. 12, no. 186.
- YAGI, S. (1931) *Ēhimeken Dōbutusi*, p. 67, no. 733; Tikami.
- KIKUTI (1931) *Toyamawan Nantaidōbutu Mokuroku*, p. 2, no. 10; Abugasima.
- GISLÉN, T. (1931) *Journal of the Faculty of Science Imperial University of Tokyo*, Sect. 4, Vol. 2, Part 4, pp. 435, 436, 441; Misaki.
- BERGENHAYN (1933) *Kungl. Svenska Vetenskapsakademiens Handlingar*, Bd. 12, No. 4, S. 39-40, Taf. 1, Fig. 12, Taf. 13, Fig. 60-67; Text-fig. 13, a-c; Misaki, Sagami.
- ASANO, H. (1933) *Bunrui Suisan Dōbutu Zusetu*, p. 252, fig. 348.
- KURODA, T. (1933) *Hukuikensan Kairui Mokuroku*, p. 179, no. 7; Nibu: Turuga; Onihu; Ōi.
- SIBA, N. (1934) *Journal of Chosen Natural History Society*, No. 18, p. 12; Tyōsen.
- KURODA, T. (1935) *Miyazakikensan Kairui Mokuroku*, p. 39, no. 6; Aosima.
- HATAKEDA (1935) *Venus*, Vol. 5, No. 4, p. 233, no. 39; Asahimura; Kamizimasotomura; Kitakizimamura, Okayama Prefecture.
- (1936) *Venus*, Vol. 6, No. 2, p. 116, no. 27; Sikaimura; Kitaauramura; Hukudamura; Yasudamura; Sakatemura; Nisimura, Syōdosima, Kagawa Prefecture.
- Liolophura japonica tessellata*
- PILSBRY (1893) *Manual of Conchology*, Vol. 14, pp. 243-244, pl. 53, figs. 45, 46; Enosima.
- (1895) *Catalogue of the Marine Mollusks of Japan*, p. 115; Enosima (STERANS).
- NIERSTRAZ (1905) *Siboga-Expedition, Monographie 48*, p. 108.
- YOKOYAMA (1931) *Catalogue of Marine, Freshwater and Land Shells of Japan*, p. 16, no. 469; Iyo.
- YAGURA (1932) *Hyōgokensa Kairui Mokuroku*, p. 20, no. 247; Tazima; Harima; Settu; Awazi.
- TAKI, IS. (1936) *Saitō Hōonkai Hakubutukan, Zihō*, No. 30, p. 1, no. 11; Akita; Yamagata; Miyagi Prefectures.

Body large, elliptical in outline; shell broad, moderately elevated, roundly arched, not carinated at the back, generally much eroded and encrusted; girdle medium in breadth, covered densely with numerous spines, more or less regularly tessellated with white and dark brown.

Head valve thick, solid, nearly semicircular in outline; apex not prominent; tegmentum ornamented with exceedingly minute granules, sparsely studded with numerous, black aesthetes on the whole surface, weakly wrinkled by indistinct furrows, giving a tendency to form radial riblets, intersected by strong concentric lines of growth; interior of the valve smooth, shining, weakly callused at about the middle, having 8 to 10 slits at the periphery; slit-lines indistinct without pores; teeth short, thick, strongly pectinated on the outer surface; posterior margin broadly reflexed.

Median valve oblong in outline, roundly arched at the jugum, slightly

sinuated at the middle of the anterior edge, much beaked but not pointed at the posterior edge; jugal area not well defined; lateral area hardly raised, scarcely defined by either a boundary rib or sculptures; tegmentum provided with numerous, fine, diagonally arranged granules on the whole surface; interior of the valve smooth, polished, with strongly elevated transverse callus at the middle; lateral and central regions deeply concave; slit small, one on each side; teeth thick, very short, pectinated on the outer surface; slit-line indistinct, without a pore; sutural laminae thick, broad, extending well forward, connected with each other by a small jugal lamina; sinus broad, deep, regularly arched.

Tail valve triangular in outline, nearly straight at the anterior edge, roundly arched on both sides; central area broad, flat, occupying a greater part of tegmentum, finely granulated likewise that of the median valve; mucro terminal, indistinct, bluntly produced posteriorly beyond and above the eaves; posterior area small, a little concave, lying under the central area; in place of insertion plate, crescentic ridge present, which is broad, flat, thick, smooth, a little wrinkled on the posterior surface, though not forming a distinct slit, becoming lower gradually passing over to the sutural lamina; central callus well developed in parallel with the crescentic ridge; central region strongly concave; sutural laminae extending remarkably forward to form a deep sinus between them, pectinated at the outer edge on the surface, bridged each other by jugal lamina; eaves broad, roughened by minute granules on the surface.

Colouration: tegmental surface of the valve without luster; blackish, generally showing a wide light stripe on each side of the black dorsal stripe; interior of the valves black, shining; sutural laminae black on both surfaces.

Girdle conspicuously varied with alternate patches of white and scorched brown or blackish, densely clothed with two kinds of numerous short, thick spines, becoming smaller toward the outer edge; upon the dark tracts of the girdle, spines reddish-brown or blackish, unicoloured or tipped with light brown or cream-white, nearly straight, obtusely pointed at the tip, much thickened at the base; upon the light tract, spines somewhat larger than the former, slightly curved, unicoloured white or light brown on the dorsal surface with dark-brownish on the ventral surface, somewhat flattened at the tip and nearly round in section at the base; marginal spine small, thin, hyaline, containing numerous minute granules, more or less pointed at the tip; hyponotum covered with minute, hyaline scales, which are coarsely striated on the surface, slightly smaller at the front

end, with squarish basal plate, near the peripheral region of this tract they are much elongated, faintly marked with a few striations.

**Radula:** central tooth remarkably long, slightly pointed, distinctly cusped at the tip; cutting edge slightly sinuated at the middle, both sides somewhat constricted; posterior end a little dilated, bilobed; basal plate rhombic shaped, situated at the middle of the tooth; centro-lateral broad, strongly cusped at the anterior edge, with a small triangular outer wing at the posterior corner, protruded posteriorly, slightly sinuated at the inner edge, basal plate broad, extending outside; major lateral thick, stout, unicuspidate, its cutting edge roundly arched, having a large wing at the inner edge of the shaft, bearing a strong cusp; outer edge protruded at the middle, broadly extended outside at the base, dorsal process well developed; inner small-lateral broadly protruded posteriorly, narrowing anteriorly; outer small-lateral thick, roundly lobed anteriorly with a small basal plate articulating with the outer edge of the preceding tooth, posterior lobe having a small process, directed outside to cover the basal part of the major uncinus; major uncinus having a broad cusp on the long stout shaft, erected from the small basal plate; inner marginal oblong in shape, having large triangular process at the middle, small denticle at the posterior edge, weakly waved at the outer edge; median marginal oval shaped, slightly bilobed at the inner edge, bluntly pointed posteriorly; outer marginal pentagonal in shape, somewhat longer than wide, shallowly sinuated at the anterior edge, protruded posteriorly.

**Size:** Length 35 mm, breadth 21 mm (type, LISCHKE).

„ 50 mm, „ 20 mm (PILSBRY, girdle excluded).

„ 36 mm, „ 26 mm (Mutsu Bay, somewhat contracted).

The largest specimen I have measured is 68 mm by 43 mm.

**Remarks:** A form of chitons collected by LINDHOLM from Hakodate, Hokkaidô was identified by SCHRENCK (1859) as *Chiton spiniger* which had been known only from tropical waters. The subsequent authors, such as SOWERBY (1930) and LELOUP (1933), quoted this locality as that of *Acanthopleura spiniger* (= *Acanthozostera gemmata*). SCHRENCK's note on this species is not altogether clear for me. This may be *Liolophura japonica* judging from its sculpture and colouration.

TAPPARONE-CANEFRI (1874) described *Chiton defilippii* from Japanese waters and distinguished it from *Chiton piceus* (= *A. gemmata*) by its peculiar features. But his diagnosis is not full enough for subsequent reference. PILSBRY examined the species and came to the conclusion that it is a synonym of *L. japonica*. His decision seems quite right.

LISCHKE (1893) described *Chiton japonicus* from Nagasaki, and PILSBRY (1893) quoted this in his manual under the genus *Liolophura* as the type and described its variety, *tessellata*, in which the girdle is much narrower and regularly tessellated with alternate patches of white and scorched brown or blackish and the spinelets are larger and more flattened than in the typical form. In observing numerous material of this species from the type locality and from other places of Japan, it was recognized that the girdle and the spinelets exhibit exceeding variation in size, colour and in width. I can not, therefore, distinguish *L. tessellata* from *L. japonica* from the features of the girdle.

The individual provided with narrow girdle, and rather small, uniformly coloured spinelets, figured by PILSBRY in plate 53, figures 41 and 42 in his manual, was considered by him as one of the typical form. It is, however, clearly distinguished from the type in all respects of the girdle.

At the same time, PILSBRY showed the type of CARPENTER's unpublished species, *Ornithochiton* (?) *caliginosus*, collected from China Sea and Hongkong, shown in figures 41-45 of plate 54 in his manual and believed to be identical with *L. japonica*, notwithstanding its sculpture of tegmentum and the most features of the girdle armatures and the insertion plate of the tail valve differ distinctly from those of *L. japonica*. It is undoubtedly different from CARPENTER's species.

HADDON (1886) reported *Acanthopleura incana* taken from Oosima, Japan during the voyage of "Challenger". NIERSTRASZ (1905) recorded 9 specimens of *L. japonica* var. *tesselata* [sic] from Molucca now in the collection of the Leyden Museum. The former of the two was identified by PILSBRY (1893) as *L. japonica*. It seems to me much proper to treat this form as identical with *L. loochooana* by its peculiar sculpture of valves, and the latter as an Australian species of the genus.

Distribution :

Pacific coast

Hokkaidô: Hakodate (SCHRENCK, Iw. TAKI) (1).

Honsyû: Miyako (3); Hirota Bay (TOBA) (4), Iwate Pref. Ôsima (5); Karakuwamura (6); Kesennuma (7); Onagawa (8); Kinkazan (9); Ayukawa (10); Amizisima (11); Watanoha (12); Matusima Bay (TAKI) (13), Miyagi Pref. Hutomi (HIRASE) (14), Tiba Pref. Uraga (TAKI) (15); Misaki (TAKI, BERGENHAYN) (16); Enosima (THIELE, STEARNS, TAKI) (17); Manazuru (TAKI) (18); Kanagawa Pref. Ôsima (YAMAMURA, K. ÔYAMA) (19); Kamizusima (MIYAZI) (29), Izu Sititô. Atami (TAKI) (21); Simoda (KANEKO) (22);

Enoura (HARA) (23); Omaezaki (A. HUZITA) (24), Sizuoka Pref.  
Wagu (T. YAMADA) (25), Mie Pref. Tanabe (Y. OKADA) (26);  
Seto (Iw. TAKI) (27); Gobô (OKAMOTO) (28); Kada (R. TANAKA)  
(29), Wakayama Pref.

Sikoku: Murotozaki (HIRO) (30); Kôti (KAMOHARA) (31); Kasiwazima  
(SUGIMOTO) (32), Kôti Pref. Yawatahama (TAKI) (33), Ehime Pref.

Kyûsyû: Saganoseki (K. TAKAGI) (34), Ôita Pref. Aosima (KURODA)  
(35), Miyazaki Pref. Natui, Sibusi Bay (MITUKURI) (36), Kagosima  
Pref.

#### Inland Sea

Honsyû: Settu (YAGURA) (37); Akasi (R. TANAKA) (38); Awazi  
(YAGURA, Z. MORITA) (39), Hyôgo Pref. Nôzizima (IWAKAWA);  
Kônosimasotomura (HATAKEDA) (40); Asahimura (HATAKEDA) (83)  
Okayama Pref. Uzina (TAKI) (41), Hiroshima Pref.

Sikoku: Siratori (Z. MORITA) (42); Syôdosima (HATAKEDA) (84)  
Kagawa Pref. Tikami (YAGI) (43); Mitu; Gogosima (TAKI) (44);  
Ehime Pref.

Kyûsyû; Siraki (K. TAKAGI) (45), Ôita Pref.

#### West coast

Kyûsyû: Akune (MITUKURI) (46), Kagosima Pref. Amakusa (BABA)  
(47), Kumamoto Pref. Nagasaki (LISCHKE, SONEHARA) (48);  
Nanatukamamura (Y. KÔNO) (49); Kurosemura (S. KINOSITA) (50);  
Sasamura (TAKAMASU) (51); Sisikimura (NISI) (52); Hirado (HIRASE)  
(53); Takasimamura (HIROYAMA) (54); Matoyama (SIRAKABE) (55);  
Kaminoura (SEGAWA) (56), Ôsima; Kisyuku (YOSIDA) (57), Gotô  
Rettô; Ituhara (EGUTI) (58); Nii (ABIRU) (59), Tusima, Nagasaki  
Pref.

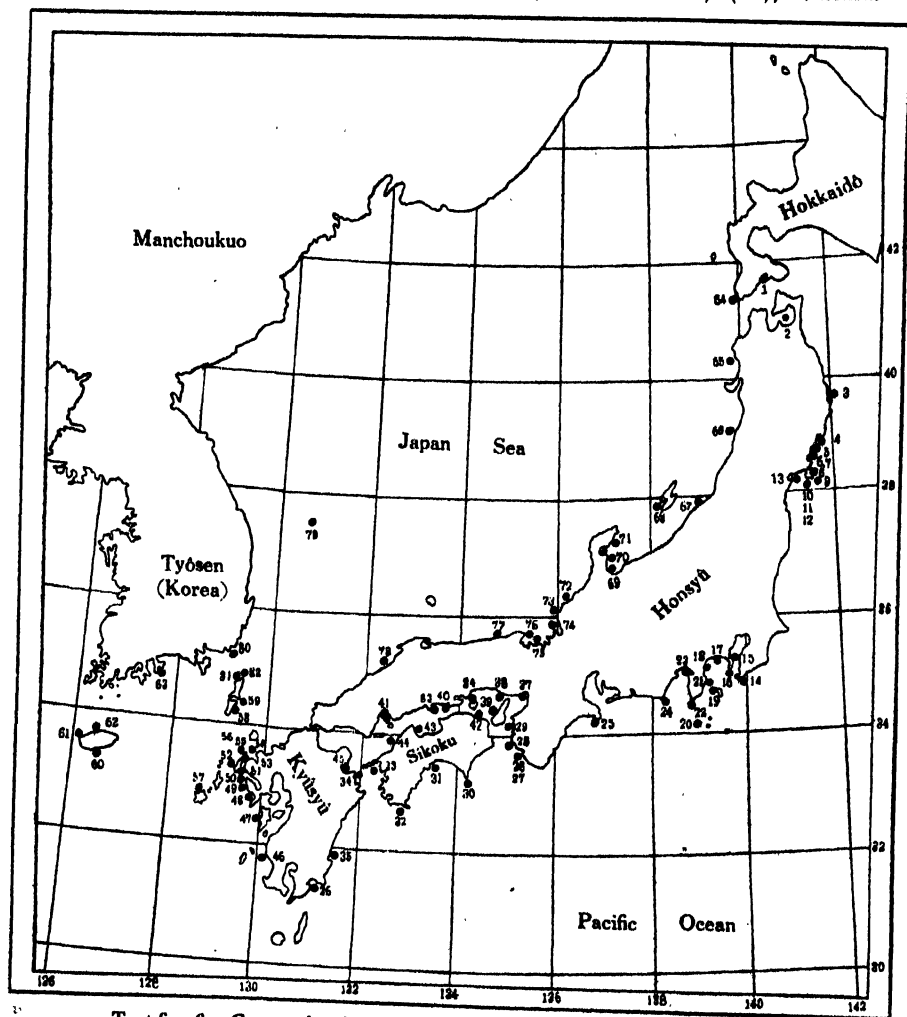
Tyôsen: Nisikiura (MAEDA, Y. YOSIDA) (60); Saisyû (OKUDA) (62);  
Zyôzanho (KURIHARA, OKUDA) (61), Saisyûtô. Reisui (OKUDA) (63);  
Zenranandô.

#### Japan Sea

Hokkaidô: Hukuyama (T. KINOSITA) (64), Hakodate Pref.

Honsyû: Mutsu Bay (2), Aomori Pref. Iwadate (TAKI) (65), Akita  
Pref. Kamo (TAKI) (66), Yamagata Pref. Niigata (EMURA) (67);  
Hutami, Sado (EMURA) (68); Niigata Pref. Husiki (HARA) (69);  
Abugasima (K. KIKUTI) (70), Toyama Pref. Usyutu (HARA) (71);  
Isikawa Pref. Sakai (HARA) (72); Nibu (73); Turuga (74); Onihu  
(75); Ôi (KURODA) (76), Hukui Pref. Tazima (YAGURA) (77) Hyôgo  
Pref. Torimura (S. TAKAGI) (78), Simane Pref.

Tyôsen: Uturyôtô (SUGIYAMA) (79); Husan (Y. YOSIDA) (80), Keisyô-nandô. Sago (ÔISI) (81): Sasuna (H. WATANABE) (82), Tusima.



Text-fig. 6. Geographical distribution of *Liolophura japonica* (LISCHE).

### Genus LUCILINA DALL 1882

#### 21. *Lucilina amanda* THIELE 1910

Pl. XV, fig. 9; Pl. XXXIII, figs. 9-13; Pl. XXXIV, figs. 5-9.

#### *Lucilina amanda*

THIELE (1910) Zoologica, Bd. 22, Heft 56, S. 97, Taf. 10, Fig. 42-50; Westküste Japans bei Tago, in einer Tiefe von 75 m.

Body about 18 mm long, 10 mm broad ; shells 7 mm in maximum width, rectangularly elevated at the jugum with rounded ridge, slightly arched on both sides, generally rose-red in colour.

Head valve ornamented with about 10 radial series of scaly granules and pits of aesthetes ; teeth long, finely pectinated on the surface ; slits 9 on the anterior margin ; interior of valve smooth, not callused in the middle, with indistinct slit-lines.

Median valve : central area ornamented with longitudinal furrows, which are more or less shorter than the length of this area except for the outermost, that barely reaches the anterior edge ; jugal area not distinctly defined, merely separable from other areas by its smoothness ; sutural laminae short, triangular, pectinated at the outer edge ; jugal articulamentum finely denticulated at the front edge ; lateral area bordered anteriorly with a series of scaly granules, posteriorly coarsely granulated, interspace between them nearly smooth, slit one on each side.

Tail valve : mucro situated a little behind the middle, distinct, not elevated ; posterior area bordered by distinct ridges, bearing radial series of aesthetes, steep, indistinctly sculptured, slits 9-11 on the posterior margin of articulamentum ; sutural laminae flat, broad, somewhat pectinated on both outer edges.

Girdle : perinotum covered sparsely with small, pointed distally ribbed spinules, measuring  $30\ \mu$  in length,  $12\ \mu$  in breadth, cylindrical spines scattered among them, about  $50\ \mu$  long,  $5\ \mu$  thick ; hyponotum armed with small scales, weakly ribbed, becoming smooth toward the periphery ; marginal spines bluntly pointed, very slightly striated, measuring  $70\ \mu$  in length,  $15\ \mu$  in thickness.

Radula : central tooth small, dilated at both ends, with distinct cusp at the tip ; centro-lateral exceedingly large, winged at the front end, never cusped, outer edge sinuated at the anterior corner, forming posteriorly a broad, angular lamella ; major lateral bears 4 broad cusps, inner wing small, with reflexed tip ; major uncinus broad, oval shaped, curved a little at the middle, stalk stout, short, with large basal plate.

Gills holobranchial, occupying about  $5/6$  the entire length of the foot, branchiae 21 on one side.

Size : Body length 18 mm, breadth 10 mm (type).

          "      "      16 mm,      "      10 mm.

Head valve 6.5 mm, second valve 5.8 mm, fourth valve 6.7 mm, tail valve 5 mm in breadth.

Remarks : THIELE described this species from the single specimen



collected by DÖDERLEIN from the west coast of Japan near Tago (Tango?) at the depth of 75 m. The specimen before me seems to be referable to this species, as they fairly agree in most features of its characters except for the borders of the lateral areas, which are not demarcated by distinct ridges in the type specimen, but only distinguishable from other areas by its peculiar sculpture. In Mutsu Bay it was dredged from the depth of about 30 m, while the type from about 75 m. The fact seems to indicate that it occurs in deep waters of southern sea, whereas in much northern locality it lives in relatively shallow waters.

Locality: Ōma, station 104; one specimen was collected by HÔZAWA, TAKATUKI and SATÔ in August, 1927, together with *Rhysoplax tectiformis*; sp. no. 2076.

Distribution:

Japan Sea

Honsyû: Mutsu Bay, Aomori Pref. Tango (THIELE), Kyôto Pref., at the depth of 75 m.

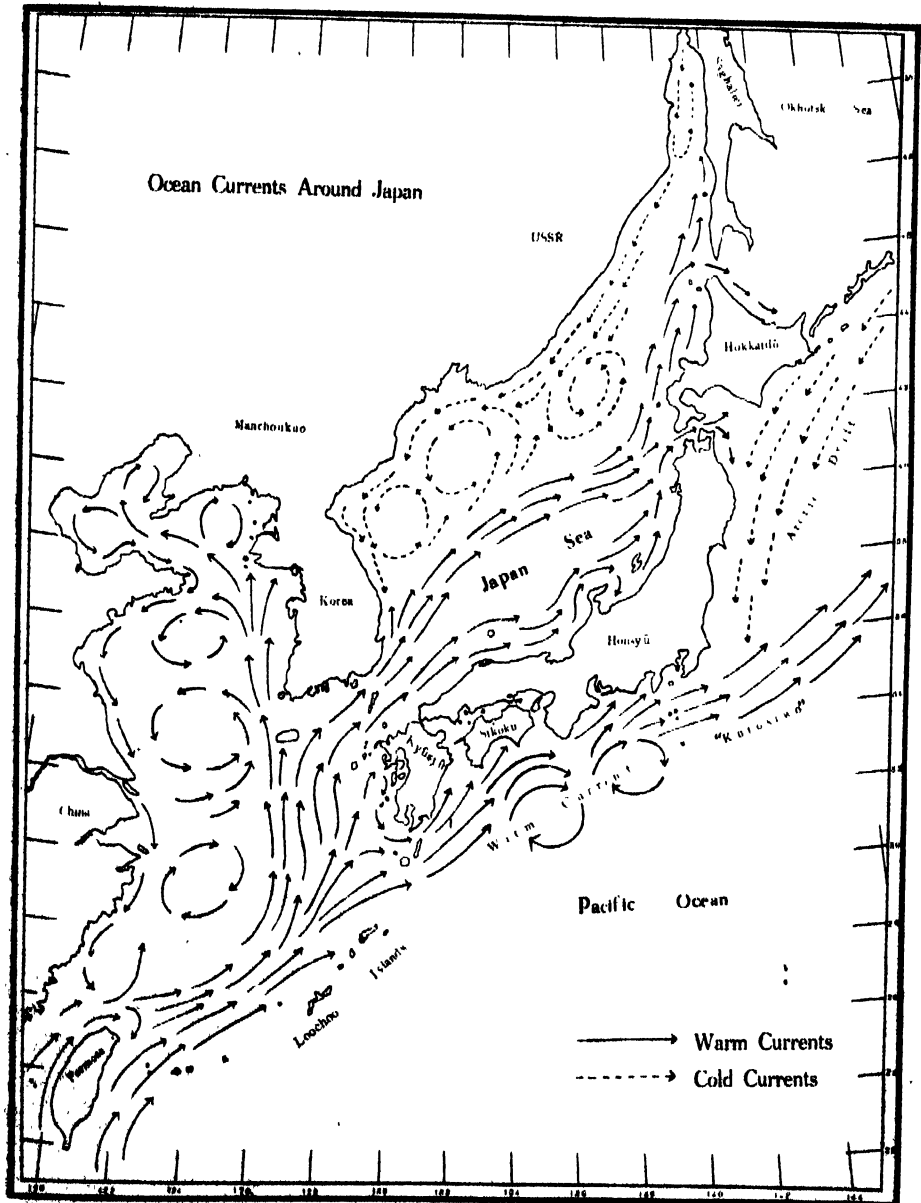
## GENERAL COMMENT ON THE CHITONS OF JAPAN

### I. INTRODUCTORY NOTE

The fauna of chitons in the northern Pacific has been investigated first by MIDDENDORFF (1847, 1851), and SCHRENCK (1867) reported several species from Alaska, the Aleutian Islands, the Okhotsk Sea and northern Japan. GOULD (1846, 1859) and DALL (1876-79, 1921) also described some species from the Aleutian and the Bering Islands. Later BERRY (1917, 1925-27, 1928) added the supplemental data to the previous results and gave a full account of the chiton fauna of these districts. Besides these authors, REEVE, TAPPARONE-CANEFRI, LISCHKE, PILSBRY, THIELE, BERGENHAYN, and myself together with my brother, described new species from the various places of Japan. The whole collection of chitons brought by the expeditions of the Challenger and the Siboga from Malay Archipelago and adjacent territories were worked out respectively by HADDON (1886) and NIERSTRASZ (1905). Australian and Neozelanic shores have long been famous for the wealth of their chiton fauna and furnished abundant material for the researches of many European authors, and a complete account concerning their fauna was given by recent workers such as ASHBY, IREADALE, HULL, MESTEYER, MACKAY.

Despite the fact that the taxonomic studies of chitons have been carried out fairly well basing upon the material collected from various parts of

the world except for the greater part of Chinese coasts, little attention has so far been directed to the problem of their distribution.



Text-fig. 7. Ocean Currents around Japan.

## II. THE FAUNAL AREAS OF CHITONS

The distribution of chitons of the western Pacific Ocean may be roughly divided in three regions; I. The first region extends from the Kurile Islands to Formosa along the entire coast of the Pacific with the Inland Sea as its subregion. II. The second region is the west coast of Kyûsyû and Korea including adjacent islands and the Gulf of Liantung and Pechili and also the Yellow Sea on the Chinese side. III. The third region includes the whole shore of the Japan Sea from the Mamiya straits to the Tusima straits, which is bounded by a line drawn through Husan and Simonoseki, embracing the northern coast of Tusima. As is noticed in other marine animals, among the factors determining the faunal region of chitons in the western Pacific, the most important may be the ocean currents. The warm current "Kurosiwo" sweeps along the eastern coasts of Luzon and Formosa and the southern coasts of Kyûsyû, Sikoku and Honsyû, giving off a branch along the western coast of Kyûsyû beyond the boundary of this region to the Japan Sea along the northern coast of western Honsyû. The Arctic drift from the Bering straits washes the entire northern coast of Japan and is checked at about the middle part of Honsyû by the warm current, flowing farther to the south and submerges under the current.

From these considerations it seems much advisable to demarcate the northern from the southern subdivision by an oblique plane formed between the two ocean currents. Both currents differ in temperature and carry faunas peculiar to them, which may be determined by isothermal lines in different level. Thus it will be found that the chitons which occur in shallow waters of the northern sea often extends to relatively deep waters of much southern localities<sup>1)</sup>. *Lucilina amanda* was dredged from Mutsu Bay at about a depth of 30 m, while from off Tango, Japan Sea, it was obtained at 75 m in depth.

Although both the western coast of the main island of Japan and the Japan Sea receive a branch of the warm current, the former has a pronounced tidal difference, while the latter has hardly any. This fact seems to influence considerably the faunal difference, working with other factors, between the two regions. *Acanthochiton rubrolineatus* thrives better on

<sup>1)</sup> A similar mode of occurrence is found in boreal-forms of gastropods: thus *Fusitriton oregonensis* was discovered in abundance at about 100 m in depth of the Kurile Islands, Saghalien and Hokkaidô, at about a depth of 300-400 m in Kumano-nada and Tosa Bay on the Pacific side and at 300 m in Toyama Bay and off Tazima in the Japan Sea.

the entire western coast, whereas *A. achates* is not found in this region, but occurs in abundance in the Japan Sea and on the Pacific coast of Honsyû, Sikoku and Kyûsyû.

The warm current has little influence on the fauna of the Inland Sea, where no peculiar genera or species of chitons are found. *Onithoplax hirasei* does not live in the Inland Sea, while it is widely distributed extending from Formosa to Matusima Bay on the Pacific coast and to Sado in the Japan Sea. A related form, *Lucilina interplicata* is met with on the Pacific coast from Formosa to the Bôsô Peninsula as well as in various localities of the Inland Sea.

It may also be mentioned here that the specimens of *Liolophura japonica* collected from the southern coasts of Kyûsyû and Sikoku have long, fairly thick calcareous spines on the girdle, while they are very delicate in those from the Inland Sea. The same thing may be said of the girdle scales of *Ischnochiton comptus* and of the tegmental granulations of *Acanthochiton rubrolineatus*.

### III. ELEMENTS OF THE CHITON FAUNA OF THE WESTERN PACIFIC

The faunal components of the western Pacific may best be considered vertically as well as horizontally.

#### (A) Horizontal distribution

##### 1. Continuous horizontal distribution.

They consist of the circumpolar, the northern Pacific, the temperate zone and the tropical forms.

a) The circumpolar elements. The genera or species which have been regarded as peculiar to the Arctic Ocean, especially to the northern Atlantic, often extend to the lower latitudes on both sides of the Pacific. Thus the European species *Tonicella marmorea* and *T. ruber* were collected from the American coast as well as from northern Japan. The latter species was found in Mutsu Bay, the southernmost limit in its distribution in Japan; *Hanleya* was also found in Sagami and Tosa Bay and certain places of the northern Japan Sea at about the depth of 200 m.

b) The northern Pacific components. As the northern Pacific genera may be mentioned *Schizoplax*, *Spongioradria*, *Mopalia*, *Placiphorella*, *Nuttallina*, *Katharina*, *Amicula*, *Cryptochiton* and *Lepidozona*. *Schizoplax*, a most striking genus of all chitons with shells divided along the median line, has a limited range in its distribution from Puget Sound to the southern coast

of Saghalien through the Aleutian Islands and the Okhotsk Sea. Of the species endemic to the area, the following live in northern Japan: *Tonicella submarmorea*, *T. lineata*, *Spongioradsia foveolata*, *Mopalia middendorffi*, *M. ciliata*, *M. wosnessenski*, *M. schrencki*, *M. hirsuta*, *Cryptochiton stelleri*, *Amicula amiculata*, *Stenoradsia lindholmi*, etc. Besides these, *Ischnoradsia hakodadensis* and *Lepidozona albrechti* range from northern Japan to Onahama on the Pacific coast and to Toyama Bay in the Japan Sea. Mutsu Bay is the southern boundary of the distribution of *Tonicella submarmorea*, *T. lineata*, *T. ruber* and *Lepidozona mertensi*.

c) The elements of the temperate zone. The chiton fauna of the area consists of a mixture of the genera characteristic of both the boreal and the tropical regions, though the majority of species are endemic and flourish evenly on the entire coast of Honsyû, Sikoku, Kyûsyû and Korea. Among the boreal genera the following species may be mentioned: *Mopalia retifera*, *Placiphorella stimpsoni*, *Lepidozona coreanica*; and among the species of the tropical groups are *Cryptoplax japonica*, *Rhyssoplax kurodai*. *Ischnochiton* and *Acanthochiton* are cosmopolitan. *Ischnochiton boninensis*, *I. comptus*, *Acanthochiton rubrolineatus*, *A. achates* may be enumerated as our representatives. Of the indigenous components which show the sporadic occurrence are *Ischnochiton melinus*, *I. paululu*, *I. mitsukurii*, *Stenoplax venustus*, *Lepidozona iyoensis*, *L. interfossa*, *L. amabilis*, *Callistochiton jacobaeus*, *Ikedaella conica*, *Rhyssoplax komaianus*, *Lucilina amanda*, *Acanthochiton dissimilis* and *Cryptoplax propior*.

d) The tropical elements. *Cryptoplax*, *Rhyssoplax*, *Notoplax*, *Acanthozostera*, *Squamopleura*, *Onithoplax* and *Lucilina* are peculiar to the tropical region. *Cryptoplax* is very rich in species in the coral reefs of northern Australia and Malay Archipelago. A few representatives of the genus occur also in Japan, *C. japonica* being found from Kyûsyû to Mutsu Bay or Hakodate. *Acanthozostera gemmata* is by far the most abundant of the littoral chitons in all the localities, where the majority of *Cryptoplax* thrives and reaches as far north as the Yaeyama Islands, which is its northern limit. *Notoplax*, comprising *N. dalli*, *N. döderleini*, *N. thielei*, *N. stewartiana* and others in our waters, may be regarded as a characteristic genus of the Indo-west-Pacific region. A striking range of meridional distribution is found in *Ischnochiton comptus*, which occurs from Malay Archipelago to Mutsu Bay or Hakodate. *Lucilina interplicata* and *Onithoplax hirasei* live from Formosa to the middle part of Honsyû. The former is found exclusively on the Pacific coasts, while *L. amanda* is the only member in the Japan Sea and the most northern species of the genus.

The latter species is on either side of Honsyû and limited in the north by a line drawn between Sado and Matusima Bay, that is very near 39° N. *Acanthochiton dissimilis* is obtained on both coasts of about the middle of Honsyû. *Acanthochiton deflippi* has the similar in range habitat with that of *Onithoplax hirasei*, though it goes as far south as the southern coast of Kyûsyû. *Liolophura loochooana* extends from Formosa and *L. caliginosa* from the Loochoo Islands to the Kii Peninsula.

## 2. Bipolarity or discontinuous meridional distribution.

In the west Pacific the genera of chitons show occasionally meridional discontinuity in their distribution, that may be called bipolarity. *Ischnoradsia hakodadensis* is found in abundance on the shores of the northern half of Japan and other species of the genus are known from Alaska and California, while four species of the genus have been recorded from Queensland, New South Wales, Victoria, South Australia and Tasmania. Three species of *Icoplax* have been reported from the eastern shores of Australia, ranging from Queensland to Tasmania and four species of the same group from New Zealand, while *I. septemcostata*, the only representative in the northern hemisphere, was collected from Sagami Bay at the depth of 150 m. Three species of *Lorica* were described from the eastern coast ranging from New South Wales to Tasmania and one from New Zealand, while a member of the genus was dredged in Tosa Bay, off Simoda and off the Bôso Peninsula at about the depth of 200 m. *Liolophura* is known from Australia and Japan, in the former locality two species have been distributed in the range between 20° and 35° S. on the eastern coast, while in the latter three species occur with the range from Formosa (22° N.) to Mutsu Bay or Hakodate Bay (41°-42° N.); *Squamopleura* ranges in its distribution between 10°-23° S. on the shores of Australia, New Caledonia, New Guinea and Malay Archipelago, while a northern representative occurs in Kôtôsyô, Formosa (22° N.); *Amphitomura* occurs on the shores of the western Indian Ocean and the Bonin Islands with an enormous intervening distance. *Onithoplax*, *Lucilina*, *Rhyssoplax* are very rich in species in the southern tropics of the Indo-west-Pacific and a few representatives occur also in our waters, from Formosa to Mutsu Bay, Honsyû.

## (B) Vertical distribution

### 1. Bathymetrical distribution.

The chitons may be grouped in three divisions according to their bathymetrical distribution, viz. the littoral, the shallow water and the abyssal

zones. The second extends between the *Laminaria* zone and the 150 m line and the third is the inhabitants of waters over 150 m.

a) The littoral zone has numerous species of heterogeneous groups in systematic position, such as *Acanthochiton*, *Ischnochiton*, *Ischnoradsia*, *Lepidozona*, *Placiphorella*, *Mopalia*, *Callistochiton*, *Cryptoplax*, *Lepidopleurus*, *Liolophura*, *Onithoplax*, *Schizoplax*. *Cryptoplax japonica* and *Lepidopleurus hirasei* are always found under stones or pebbles near the low water mark, *Schizoplax brandti* under rocks or on the leaves of *Zostera* at fairly low tides.

b) The shallow water division. Notwithstanding its abyssal characters, a few members of *Lepidopleurus* are occasionally found in shallow water. *L. latidens* was dredged from 2-3 m in Sagami Bay. *L. hakodatensis* and *L. assimilis* from the *Laminaria* zone and from the depth of 30 m. *Tonicella submarmorea*, *T. lineata*, *Spongioradsia foveolata*, *Lepidozona mertensi*, *L. iyoensis*, *Ischnochiton melinus* and *I. paululus* occur at about the depth of 20-50 m. *Lucilina amanda* was collected from 75 m in depth, while *L. interplicata* from the *Laminaria* zone. *Lepidozona interfossa* and *L. amabilis* were taken from 80 m and *Thaumastochiton* from 100 m in Sagami Bay.

c) The abyssal division. As the majority of chitons are littoral, the deep-water forms are not many. To the abyssal division belong the genera *Lepidopleurus* and *Hanleya*. *L. aequispinus* occurs at 300 m, *L. japonica* at 160 m, *L. diomedae* at 500 m; *Hanleya* at 200 m; *Lepidozona pilsbryana* at 400 m; *L. pallida* at 600 m.

## 2. Ontogenic migration.

Any one who tries to collect chitons at the shore, will soon find the fact that young ones are very rare there. So far no adequate explanation to account for this fact has been put forth. Neither has anybody full account of where and how chitons spend their young stages. The larvae of chitons are of course free swimming. Then they go down from the surface to their favorable depths to begin their bottom life. Afterward they gradually migrate toward the shore only to find at length their congenial home at the littoral zone. The following evidence seems to support the above account. Small specimens of *Ischnochiton comptus*, measuring 6-10 mm in length, were dredged at the depth of 20-30 m. The juvenile forms of similar sizes of *Lepidozona coreanica*, *L. albrechti*, *Ischnoradsia hakodadensis*, *Acanthochiton rubrolineatus*, *A. achates*, *A. defilippi*, *Onithoplax hirasei* and *Rhyssoplax kurodai* were also found at the depth of 20-80 m.

On the whole tendency has been noticed that the nearer the shore, the larger the specimens become. In his "Notes on West American Chitons" BERRY (1917) states about *Tonicella lineata* that "it is interesting to note that all the dredged specimens<sup>2)</sup> run very much smaller than those taken between tides. It may be mentioned also that whereas there are no large specimens among the dredged material, I have on the other hand seen no smaller ones from the shore". And my opinion seems to be substantiated by the above remarks. However it is certain that the deep-water forms do not change the habitat during their growth period as their littoral congeners do, since specimens of various ages can be dredged from one and the same depth.

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<sup>2)</sup> It was dredged from the depth of 10 to 30 fathoms at Forrester Island, Alaska.



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## EXPLANATION OF THE PLATES

## PLATE XIV.

- Fig. 1. *Lepidopleurus hakodatensis* THIELE, length 9 mm.  
Fig. 2. *Lepidopleurus assimilis* THIELE, length 8 mm.  
Fig. 3. *Tonicella ruber* (LINNÉ), length 6.5 mm.  
Fig. 4. *Spongioradsia foveolata*, nov. sp., length 6 mm.  
Fig. 5. *Ischnochiton comptus* (GOULD), length 23 mm.  
Fig. 6. *Lepidozona mertensi* (MIDDENDORFF), length 17 mm.  
Fig. 7. *Lepidozona coreanica* (REEVE), length 35 mm.  
Fig. 8. *Lepidozona albrechti* (SCHRENCK), length 37 mm.  
Fig. 9. *Lepidozona albrechti* (SCHRENCK), length 45 mm.  
Fig. 10. *Ischnochiton comptus* (GOULD), length 16 mm.  
Fig. 11. *Mopalia hirsuta*, nov. sp., length 9 mm.  
Fig. 12. *Tonicella lineata* (WOOD), length 16 mm.  
Fig. 13. *Placiphorella stimpsoni* (GOULD), length 25 mm.  
Fig. 14. *Lepidozona albrechti* (SCHRENCK), length 47 mm.  
Fig. 15. *Cryptoplax japonica* PILSBRY, length 30 mm.  
Fig. 16. *Tonicella submarmorea* MIDDENDORFF, length 6.5 mm.  
Fig. 17. *Placiphorella stimpsoni* (GOULD), length 46 mm.

## PLATE XV.

- Fig. 1. *Acanthochiton rubrolineatus* (LISCHKE), length 27 mm.  
Fig. 2. *Acanthochiton achates* (GOULD), length 27 mm.  
Fig. 3. *Liolophura japonica* (LISCHKE), length 36 mm.  
Fig. 4. *Rhyssoplax tectiformis*, nov. sp., length 9 mm.  
Fig. 5. *Rhyssoplax kurodai* (Is. TAKI et Iw. TAKI), length 17 mm.  
Fig. 6. *Ischnochiton comptus* (GOULD), length 18 mm.  
Fig. 7. *Ischnochiton comptus* (GOULD), length 23 mm.  
Fig. 8. *Ischnoradsia hakodadensis* ("CARPENTER" PILSBRY), length 32 mm.  
Fig. 9. *Lucikina amanda* THIELE, length 16 mm.  
Fig. 10. *Ischnochiton paululus*, nov. sp., length 5 mm.

## PLATE XVI.

- Figs. 1-4, 6-8, 14. *Lepidopleurus hakodatensis* THIELE.  
1. Front view of the 4th valve, breadth 4 mm.  
2. Marginal spinules, length 70  $\mu$ -76  $\mu$ .  
3. Dorsal view of the 4th valve, breadth 4 mm.  
4. Tail valve, breadth 3.5 mm.  
6. Girdle spines, length 120  $\mu$ -130  $\mu$ ; thickness 16  $\mu$ -24  $\mu$ .

- 7. Peripheral scales of hyponotum, length 100–120  $\mu$ ; breadth 20–30  $\mu$ .
- 8. Scales of hyponotum, length 48–52  $\mu$ ; breadth 26–30  $\mu$ .
- 14. Tegmental surface.  $\times 65$

Figs. 5, 9–13, 15. *Lepidopleurus assimilis* THIELE.

- 5. Hyponotum scales, length 60–90  $\mu$ ; breadth 30–40  $\mu$ .
- 9. Head valve, breadth 2.7 mm.
- 10. Tail valve, breadth 2.8 mm.
- 11. Front view of the 4th valve, breadth 3.5 mm.
- 12. Dorsal view of the 4th valve.
- 13. Interior of the 4th valve.
- 15. Sculpture of tegmental surface.  $\times 50$

#### PLATE XVII.

Figs. 1–4. *Tonicella ruber* (LINNÉ).

- 1. Hyponotum scales.  $\times 600$
- 2. Radula.  $\times 400$
- 3. Marginal spinal.  $\times 600$
- 4. Girdle scales.  $\times 600$

Fig. 5. Marginal spines of *Lepidopleurus assimilis* THIELE, length 156–165  $\mu$ ; thickness 21–23  $\mu$ .

Figs. 6–8. *Lepidopleurus hakodatensis* THIELE.

- 6, 7. Girdle scales, 72–84  $\mu$  in length; 45–46  $\mu$  in breadth.
- 8. Radula.  $\times 300$

Figs. 9–11. *Lepidopleurus assimilis* THIELE.

- 9. Girdle scales, length 80–86  $\mu$ ; breadth 50–55  $\mu$ .
- 10. do.
- 11. Radula.  $\times 500$

#### PLATE XVIII.

Figs. 1–5. *Tonicella ruber* (LINNÉ).

- 1. Head valve, breadth 2.3 mm.
- 2. Fourth valve, front view, divergency 98°.
- 3. Tegmental surface of the 4th valve.
- 4. Fourth valve, dorsal view, breadth 3 mm.
- 5. Tail valve, breadth 1.8 mm.

Figs. 6–8. *Tonicella submarmorea* (MIDDENDORFF).

- 6. Spine of girdle.  $\times 250$
- 7. Scales of hyponotum.  $\times 250$
- 8. Girdle scales.  $\times 600$

Figs. 9–15. *Tonicella lineata* (WOOD).

- 9. Head valve, breadth 6 mm.
- 10. Fourth valve, front view, breadth 9 mm.
- 11. Spinules of perinotum, length 40–60  $\mu$ ; breadth 12–20  $\mu$ .
- 12. Fourth valve, dorsal view.
- 13. Interior of the 4th valve.
- 14. Hyponotum scales, length 70  $\mu$ ; breadth 30  $\mu$ .
- 15. Tail valve, breadth 5 mm.

## PLATE XIX.

Figs. 1, 3-8. *Tonicella submarmorea* (MIDDENDORFF).

1. Marginal spicule.  $\times 600$
3. Radula.  $\times 200$
4. Spine of girdle.  $\times 460$
5. Fourth valve, dorsal view, breadth 3 mm.
6. Interior of the same valve.
7. Front view of the same, divergency  $104^{\circ}$ .
8. Tegmental surface of the median valve.  $\times 50$

Fig. 2. *Tonicella lineata* (WOOD), radula.  $\times 200$

## PLATE XX.

Figs. 1-10. *Spongioradsia foveolata*, nov. sp.

1. Interior of tail valve.  $\times 30$
2. Head valve.  $\times 30$
3. Interior of head valve.
4. Scales of hyponotum.  $\times 1000$
5. Tail valve.  $\times 30$
6. Left side of tail valve.  $\times 30$
7. Fourth valve.  $\times 30$
8. Interior of the fourth valve.
9. Marginal spicules.  $\times 1000$
10. Spines of perinotum.  $\times 250$

## PLATE XXI.

Fig. 1. *Spongioradsia foveolata*, nov. sp., radula.  $\times 450$

Figs. 2, 4-6. *Mopalia hirsuta*, nov. sp.

2. Radula.  $\times 250$
4. Marginal spines.  $\times 400$
5. Scales of hyponotum.  $\times 400$
6. Scales of perinotum.  $\times 700$

Figs. 3, 7-9. *Placiphorella stimpsoni* (GOULD).

3. Marginal spines,  $130\ \mu$ ,  $160\ \mu$  in length.
7. Spinules of perinotum, length  $30\ \mu$ .
8. Hyponotum spinules, length 80, 95,  $110\ \mu$ .
9. Radula.  $\times 150$

## PLATE XXII.

Figs. 1-6. *Mopalia hirsuta*, nov. sp.

1. Head valve.  $\times 18$
2. Fourth valve, front view.  $\times 18$
3. Fourth valve, dorsal view.  $\times 18$
4. Fourth valve, interior.  $\times 18$
5. Tail valve.  $\times 18$

6. Interior of tail valve.  $\times 18$
- Figs. 7-15. *Placiphorella stimpsoni* (GOULD).
7. Tail valve, breadth 7 mm.
8. Interior of tail valve.
9. Left side of tail valve.
10. Part of bristle of perinotum.  $\times 250$
11. Head valve, breadth 10.7 mm.
12. Interior of head valve, breadth 10.7 mm.
13. Fourth valve, front view, breadth 12.5 mm.
14. Same, dorsal view.
15. Interior of 4th valve.

## PLATE XXIII.

- Figs. 1-6. *Acanthochiton rubrolineatus* (LISCHKE).
1. Head valve, breadth 4.8 mm.
2. Tail valve, breadth 4.7 mm.
3. Fourth valve, front view, breadth 5.5 mm.
4. Hyponotum spinules, length 100  $\mu$ , 120  $\mu$ .
5. Fourth valve, dorsal view.
6. Spines of girdle, length 120  $\mu$ , 320  $\mu$ , 350  $\mu$ .
- Figs. 7-11. *Acanthochiton achates* (GOULD).
7. Head valve, same magnification as fig. 1.
8. Tail valve, same magnification as fig. 2.
9. Hyponotum spinules, length 60  $\mu$ , 120  $\mu$ .
10. Fourth valve, dorsal view.
11. do., front view.
- Figs. 12-13. *Mopalia hirsuta*, nov. sp.
12. Seta of perinotum.  $\times 60$
13. Bristle of seta.  $\times 1000$

## PLATE XXIV.

- Figs. 1, 2. *Acanthochiton rubr lineatus* (LISCHKE).
1. Radula.  $\times 100$
2. Spicules of hair-tuft, length 1.5 mm, 1 mm.
- Figs. 3-7. *Acanthochiton achates* (GOULD).
3. Radula.  $\times 100$
4. Marginal spine, length 540  $\mu$ .
5. Large spine of perinotum, length 490  $\mu$ .
6. Small spines of perinotum, length 70  $\mu$ , 120  $\mu$ .
7. Spicules of hair-tuft, length 1.2 mm, 1.8 mm.
- Figs. 8, 9. *Cryptoplax japonica* PILSBRY.
8. Fourth valve, front view.
9. Radula.  $\times 100$

## PLATE XXV.

- Figs. 1-5. *Cryptoplax japonica* PILSBRY.

1. Head valve.
  2. Second valve.
  3. Fourth valve.
  4. Tail valve, side view.
  5. Same, dorsal view.
- Figs. 6-8. *Ischnochiton paululus*, nov. sp.
6. Marginal spine.  $\times 600$
  7. Marginal scales.  $\times 1000$
  8. Marginal spinules.  $\times 1000$
- Figs. 9-16. *Ischnochiton comptus* (GOULD).
9. Large marginal spines.  $\times 600$
  10. Marginal spinules.  $\times 600$
  11. Small marginal spines.  $\times 600$
  12. Head valve, breadth 6.8 mm.
  13. Fourth valve, front view, breadth 8.5 mm.
  14. Fourth valve, dorsal view.
  15. Tail valve, breadth 6.5 mm.
  16. Hyponotum scale.  $\times 650$
- Figs. 17, 18. *Acanthochiton rubrolineatus* (LISCHKE).
17. Marginal spine, length 430  $\mu$ .
  18. Spines of perinotum, length 33  $\mu$ , 18  $\mu$ .
- Figs. 19-21. *Cryptoplax japonica* PILSBRY.
19. Spines of hyponotum, length 70  $\mu$ , 77  $\mu$ .
  20. Spines of perinotum, length 120  $\mu$ , 330  $\mu$ .
  21. Marginal spine, length 500  $\mu$ .

#### PLATE XXVI.

- Figs. 1-5. *Ischnoradsia hakodadensis* ('CARPENTER' PILSBRY).
1. Head valve, breadth 7.8 mm; body length 22 mm.
  2. Fourth valve, front view, breadth 9.6 mm.
  3. Same, dorsal view.
  4. Same, interior.
  5. Tail valve, breadth 7.9 mm.
- Figs. 6-12. *Ischnochiton paululus*, nov. sp.
6. Scales of perinotum.  $\times 600$
  7. Tail valve, dorsal view, breadth 2.8 mm.
  8. Same, left side.
  9. Hyponotum scale.  $\times 1000$
  10. Peripheral hyponotum scale.  $\times 1000$
  11. Fourth valve, front view, breadth 3.6 mm.
  12. Peripheral scales of perinotum.  $\times 1000$

#### PLATE XXVII.

- Figs. 1-5. *Ischnoradsia hakodadensis* ('CARPENTER' PILSBRY).
1. Radula.  $\times 150$
  2. Marginal scale of hyponotum.  $\times 600$

- 3. Marginal spinules.  $\times 600$
- 4. Scales of hyponotum.  $\times 600$
- 5. Marginal spine.  $\times 600$
- Figs. 6, 7. *Ischnochiton comptus* (GOULD).
- 6. Radula.  $\times 200$
- 7. Major uncinus, major lateral.  $\times 250$
- Figs. 8, 9. *Ischnochiton paululus*, nov. sp.
- 8. Cusp.  $\times 1000$
- 9. Central part of radula.  $\times 1000$

## PLATE XXVIII.

- Figs. 1-12. *Lepidozona coreanica* (REEVE).
- 1. Head valve.
- 2. Second valve.
- 3. Fourth valve, dorsal view.
- 4. Interior of the same.
- 5. Same, front view.
- 6. Tail valve.
- 7. Same, interior.
- 8. Same, left side.
- 9-11. Small marginal spines, length 100  $\mu$ , 102  $\mu$ , 115  $\mu$ .
- 12. Tegmental surface of pleural area.
- Figs. 13-18. *Lepidozona albrechti* (SCHRENCK).
- 13. Marginal spinules.  $\times 600$
- 14. Tegmental surface of the second valve of juvenile specimen, breadth 6.5 mm.
- 15. Head valve, breadth 12.5 mm.
- 16. Tail valve, breadth 11 mm.
- 17. Same, left side.
- 18. Same, interior.
- Figs. 19, 20. *Ischnoradsia hakodadensis* ('CARPENTER' PILSBRY).
- 19. Scale of perinotum, apical view.
- 20. do, dorsal view.

## PLATE XXIX.

- Figs. 1-6. *Lepidozona mertensi* (MIDDENDORFF).
- 1. Head valve, breadth 6.5 mm.
- 2. Fourth valve, front view.
- 3. Scales of hyponotum.  $\times 250$
- 4. Interior of the 2nd valve, breadth 7.5 mm.
- 5. Fourth valve, breadth 8 mm.
- 6. Tail valve, breadth 5.5 mm.
- Figs. 7-9. *Lepidozona albrechti* (SCHRENCK).
- 7. Fourth valve, front view, divergency 130'.
- 8. Same, dorsal view, breadth 15 mm.
- 9. Scales of hyponotum.  $\times 250$
- Fig. 10. *Lepidozona coreanica* (REEVE). Scales of hyponotum, 30  $\mu \times 80 \mu$ .



Figs. 11-16. *Ischnochiton comptus* (GOULD).

11-13. Scales of perinotum.  $\times 150$

14-16. Peripheral scales of perinotum.  $\times 700$

Fig. 17. *Lepidozona albrechti* (SCHRENCK), interior of the 4th valve, breadth 15 mm

### PLATE XXX.

Figs. 1-5. *Lepidozona coreanica* (REEVE).

1. Large marginal spine.  $\times 200$

2. Perinotum scale of juvenile specimen, breadth 30  $\mu$ .

3. Radula.  $\times 150$

4. Perinotum scale of full grown specimen, side view, height 280  $\mu$ .

5. Same, dorsal view, breadth 280  $\mu$ .

Figs. 6-9. *Lepidozona mertensi* (MIDDENDORFF).

6. Marginal spines.  $\times 400$

7. Large marginal spine.  $\times 400$

8. Marginal spinules.  $\times 400$

9. Radula.  $\times 150$

Figs. 10-13. *Lepidozona albrechti* (SCHRENCK).

10. Large marginal spine.  $\times 230$

11. Cusp of major lateral of full grown specimen.  $\times 150$

12. Cusp of major lateral of juvenile specimen.  $\times 150$

13. Radula.  $\times 150$

### PLATE XXXI.

Figs. 1-5. *Lepidozona albrechti* (SCHRENCK).

1. Perinotum scale of full grown specimen, 270  $\mu \times 310 \mu$ .

2. do., 450  $\mu \times 740 \mu$ .

3. Peripheral scale of perinotum of juvenile specimen, 140  $\mu \times 220 \mu$ .

4. Perinotum scale of full grown specimen, apical view, breadth 450  $\mu$ .

5. Perinotum scale of juvenile specimen, 140  $\mu \times 200 \mu$ .

Figs. 6, 7. *Lepidozona coreanica* (REEVE).

6. Marginal spinules.  $\times 600$

7. Perinotum scale of juvenile specimen, 130  $\mu \times 135 \mu$ .

Fig. 8. *Rhysoplax tectiformis*, nov. sp., radula.  $\times 300$

Figs. 9, 10. *Lepidozona mertensi* (MIDDENDORFF).

9. Perinotum scale.  $\times 400$

10. do., apical view.  $\times 400$

Fig. 11. *Rhysoplax kurodai* (Is. TAKI et Iw. TAKI), radula.  $\times 150$

### PLATE XXXII.

Figs. 1-7. *Rhysoplax kurodai* (Is. TAKI et Iw. TAKI).

1. Head valve, breadth 5.3 mm.

2. Second valve, front view, breadth 5.8 mm.

3. Fourth valve, dorsal view, breadth 6.5 mm.

4. Tail valve, breadth 4.7 mm.

- 5. Scales of girdle.  $\times 200$
- 6. Marginal spines.  $\times 250$
- 7. Scales of hyponotum.  $\times 250$

Figs. 8-13. *Rhysoplax tectiformis*, nov. sp.

- 8. Head valve, breadth 3.25 mm.
- 9. Second valve, front view, breadth 3.7 mm.
- 10. Third valve, dorsal view, breadth 4 mm.
- 11. Tail valve, breadth 2.8 mm.
- 12. Scales of hyponotum.  $\times 600$
- 13. Marginal spine.  $\times 600$
- 14. Scales of girdle.  $\times 350$

Figs. 15-16. *Liolophura japonica* (LISCHKE).

- 15. Peripheral scale of hyponotum.  $\times 300$
- 16. Hyponotum scale.  $\times 300$

Fig. 17. *Lepidazona albrechti* (SCHRENCK), marginal spines.  $\times 600$

#### PLATE XXXIII.

Figs. 1-8. *Liolophura japonica* (LISCHKE).

- 1. Tail valve, dorsal view, breadth 11 mm.
- 2. do., left side.
- 3. do. interior.
- 4. Head valve, breadth 13 mm.
- 5. Interior of head valve.
- 6. Fourth valve, front view, breadth 15 mm.
- 7. Same, dorsal view.
- 8. Interior of the same.

Figs. 9-13. *Lucilina amanda* THIELE.

- 9. Second valve, front view, breadth 5.8 mm.
- 10. Fourth valve, dorsal view, breadth 6.7 mm.
- 11. Spines of perinotum.  $\times 1000$
- 12. Head valve, breadth 5.6 mm.
- 13. Tail valve, breadth 5 mm.

#### PLATE XXXIV.

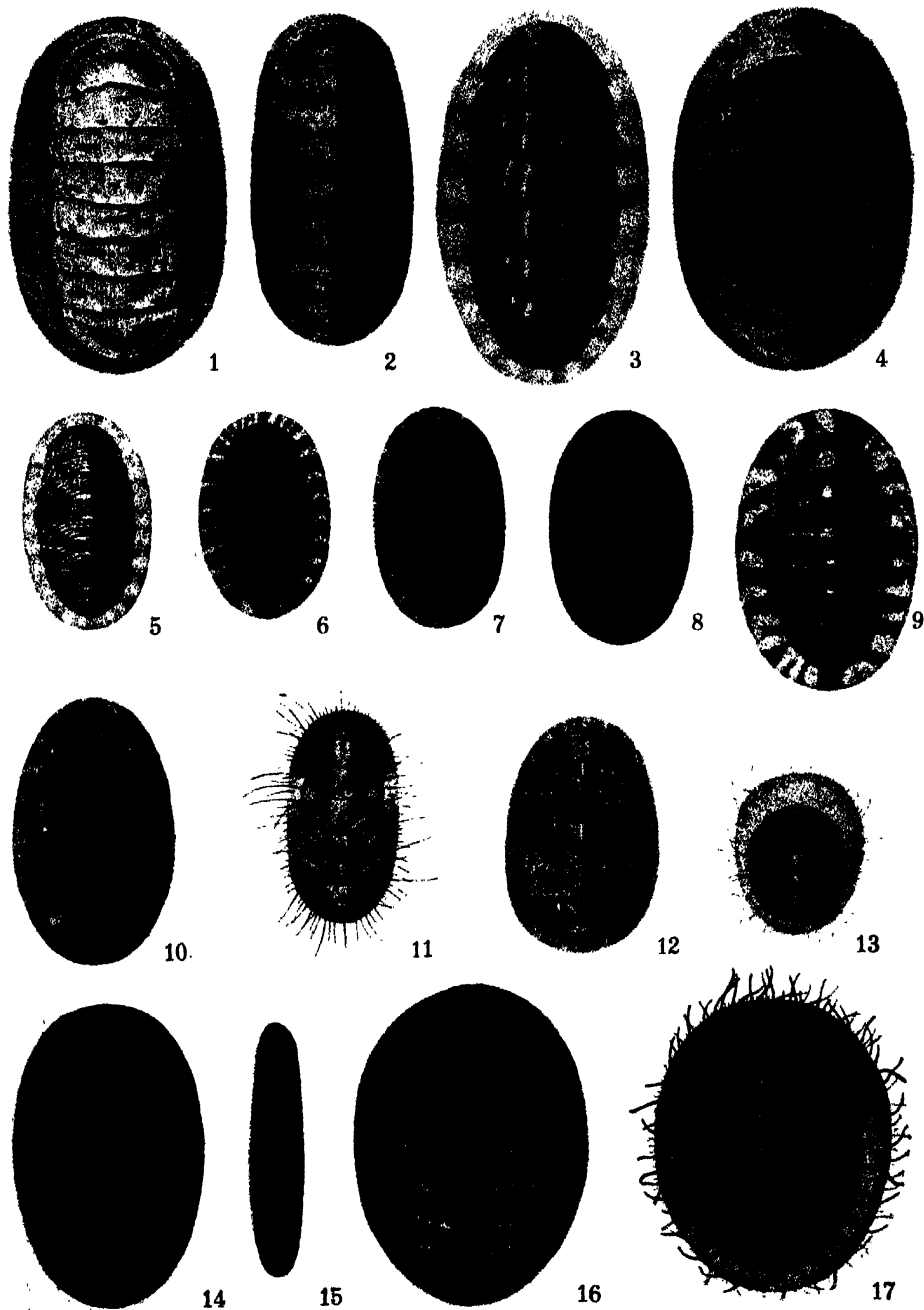
Figs. 1-4. *Liolophura japonica* (LISCHKE).

- 1. Marginal spines.  $\times 250$
- 2. Cusp of major lateral.  $\times 150$
- 3. Radula.  $\times 150$
- 4. Calcareous spines of perinotum.  $\times 40$

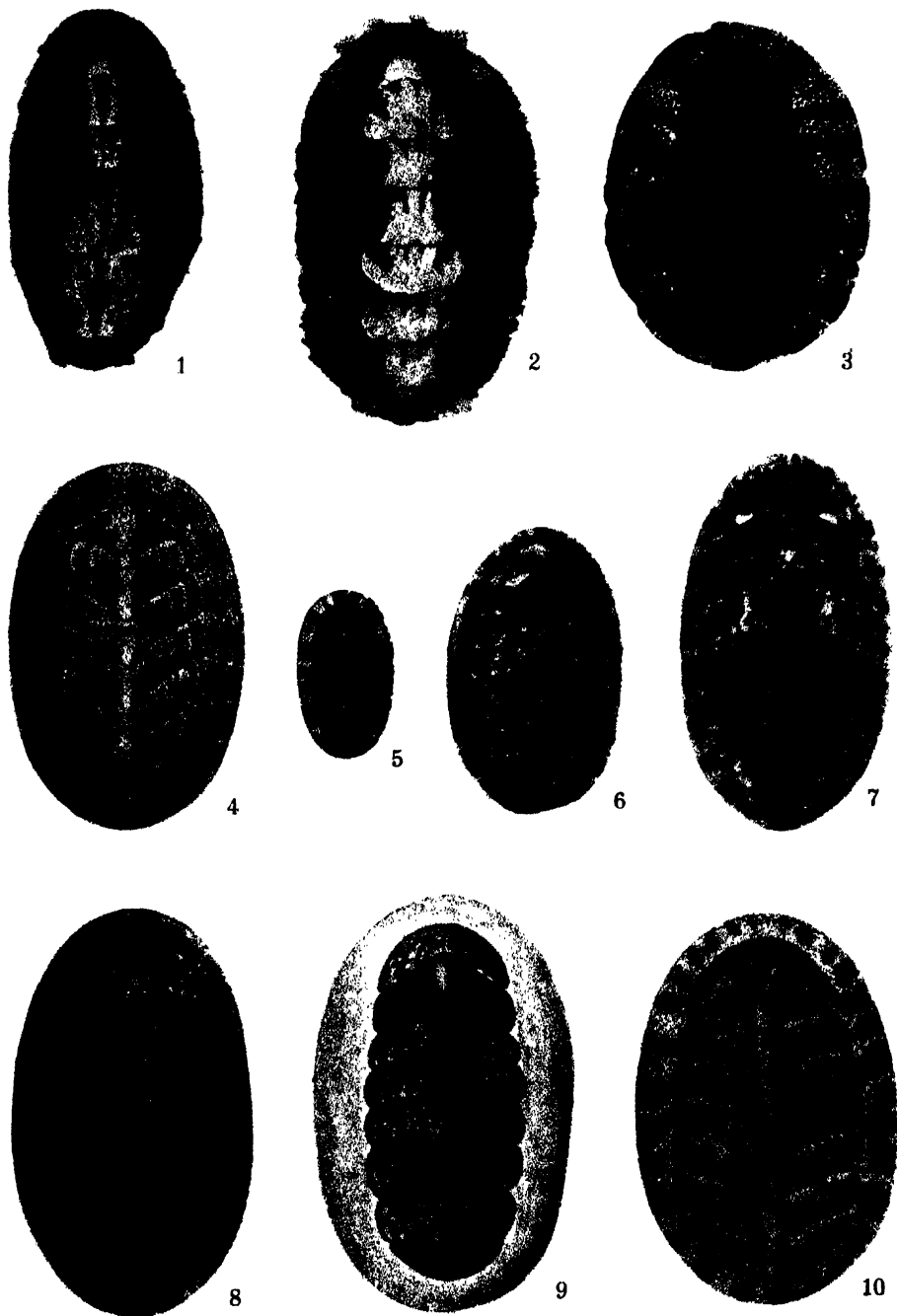
Figs. 5-9. *Lucilina amanda* THIELE.

- 5. Spinules of perinotum.  $\times 1000$
- 6. Peripheral part of radula, including inner small-lateral, outer small-lateral, major uncinus, inner marginal.  $\times 250$
- 7. Cusp of major lateral.  $\times 250$
- 8. Central part of radula.  $\times 250$
- 9. Marginal spine.  $\times 600$



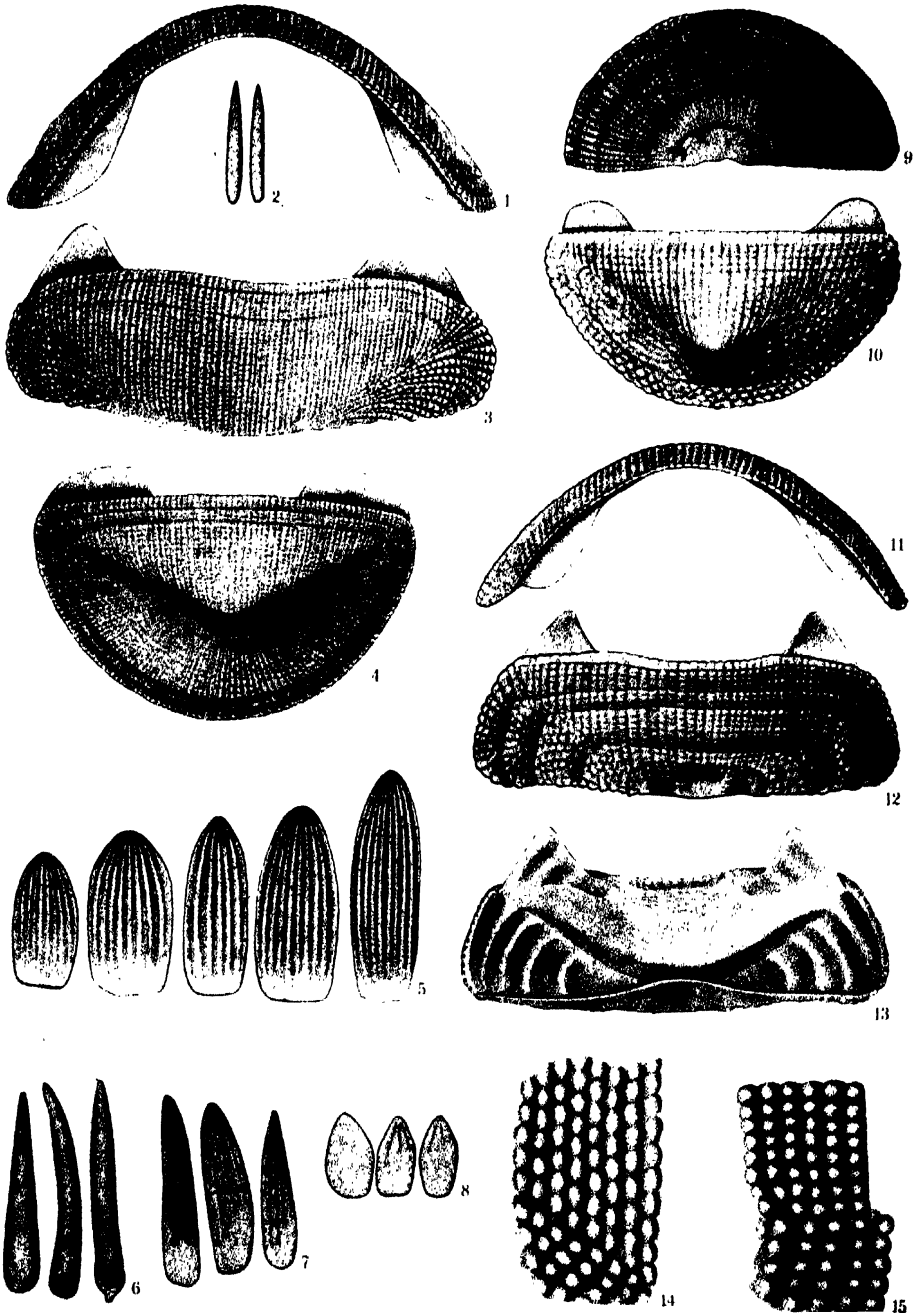






I. TAKI: Chitons of Mutsu Bay.

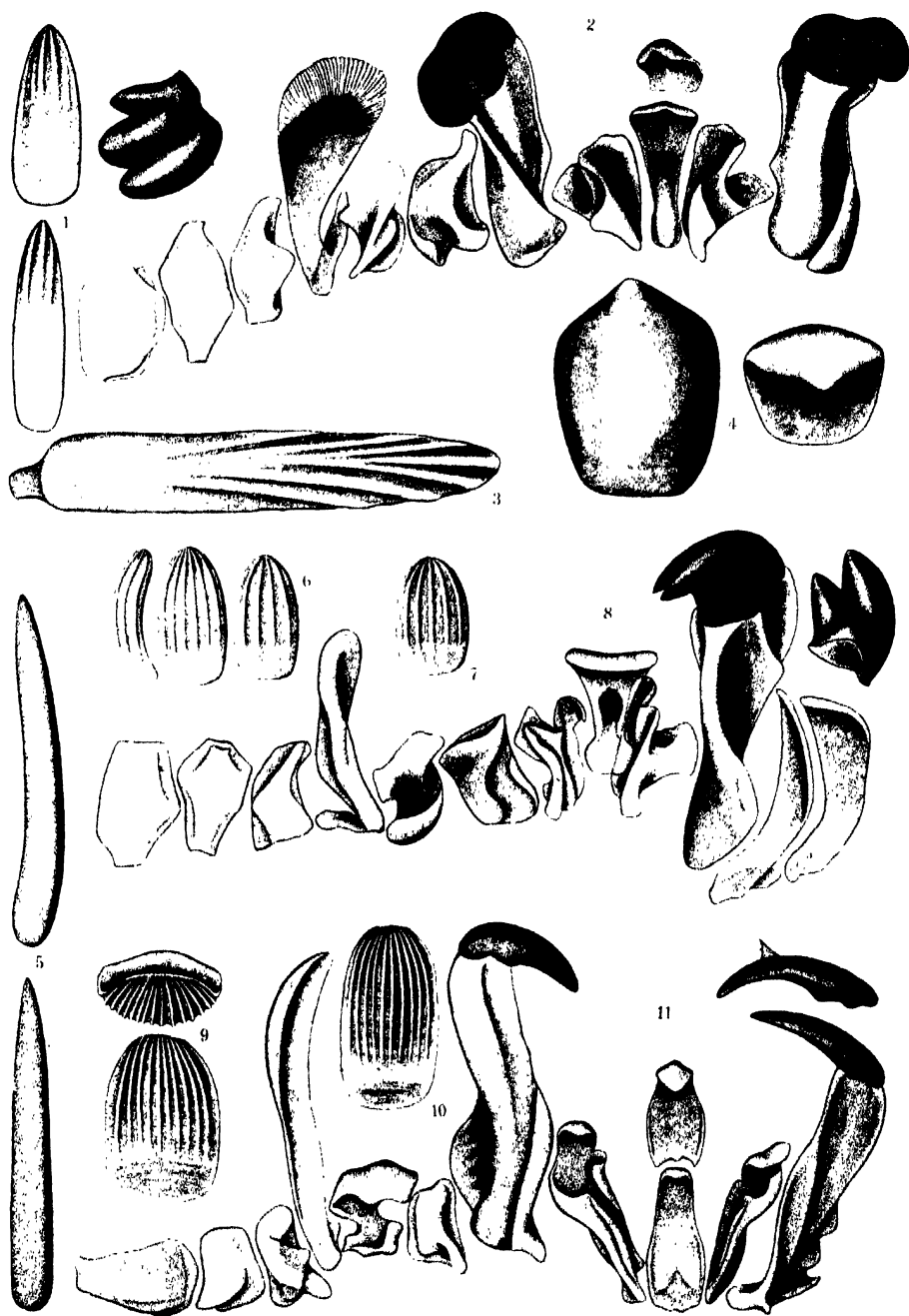




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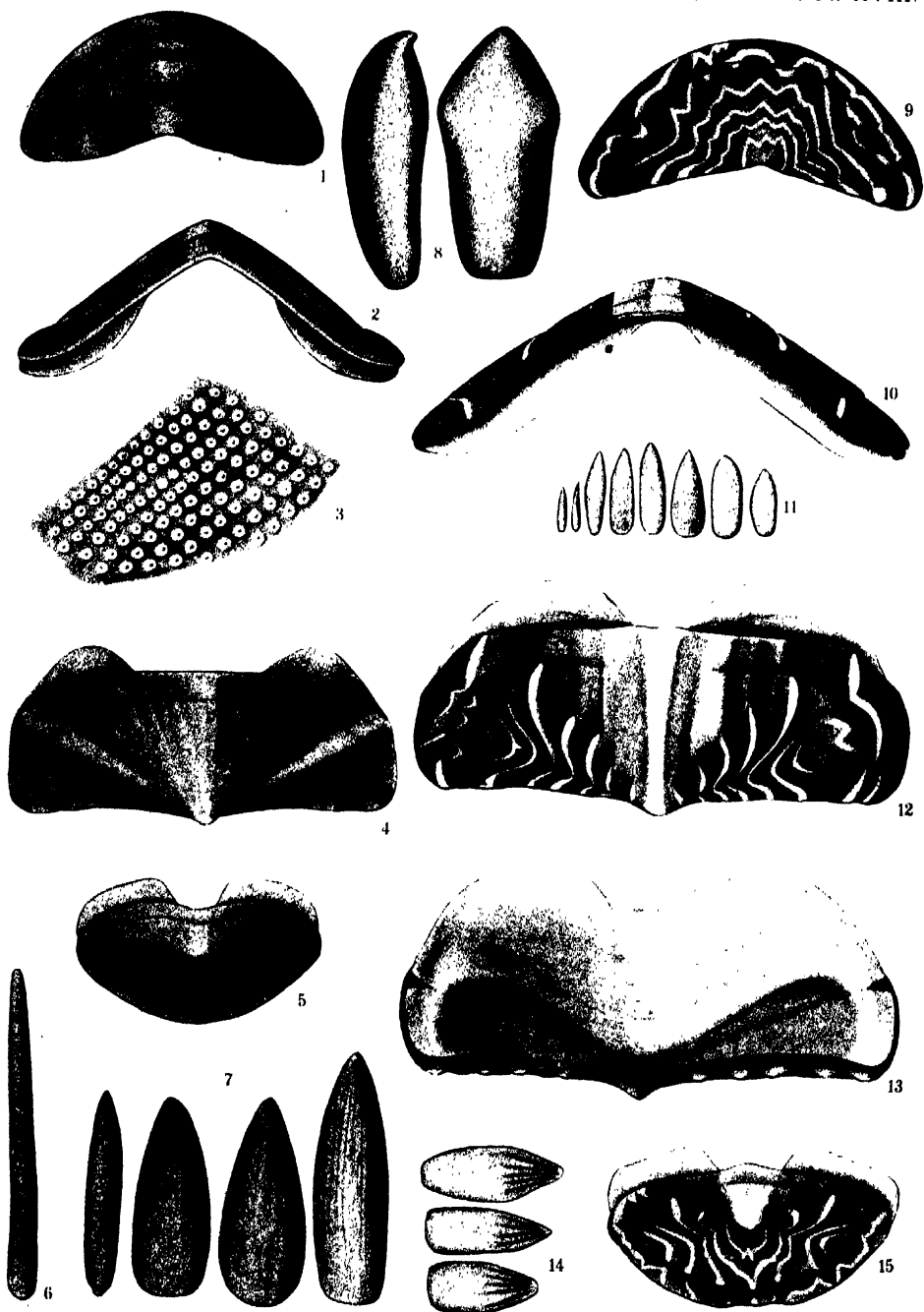






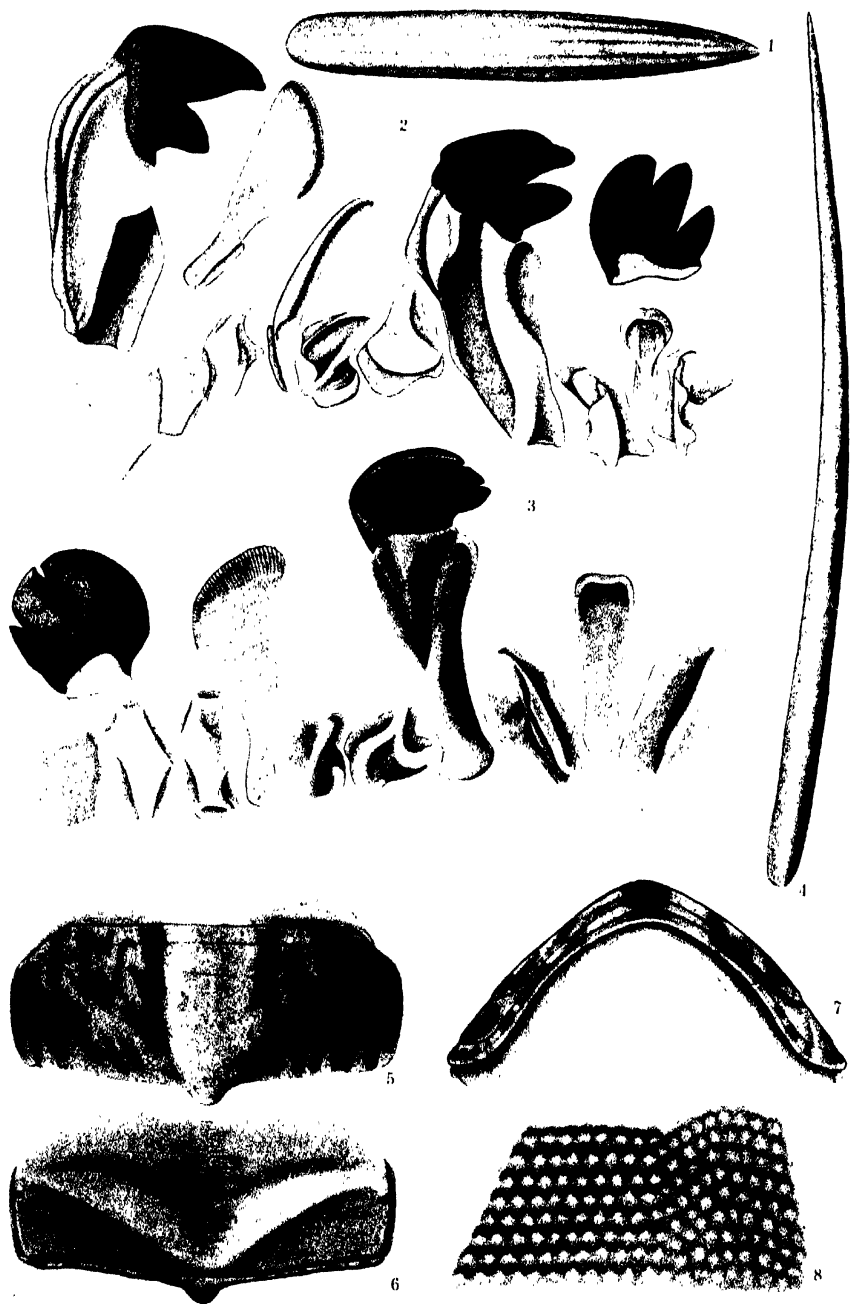
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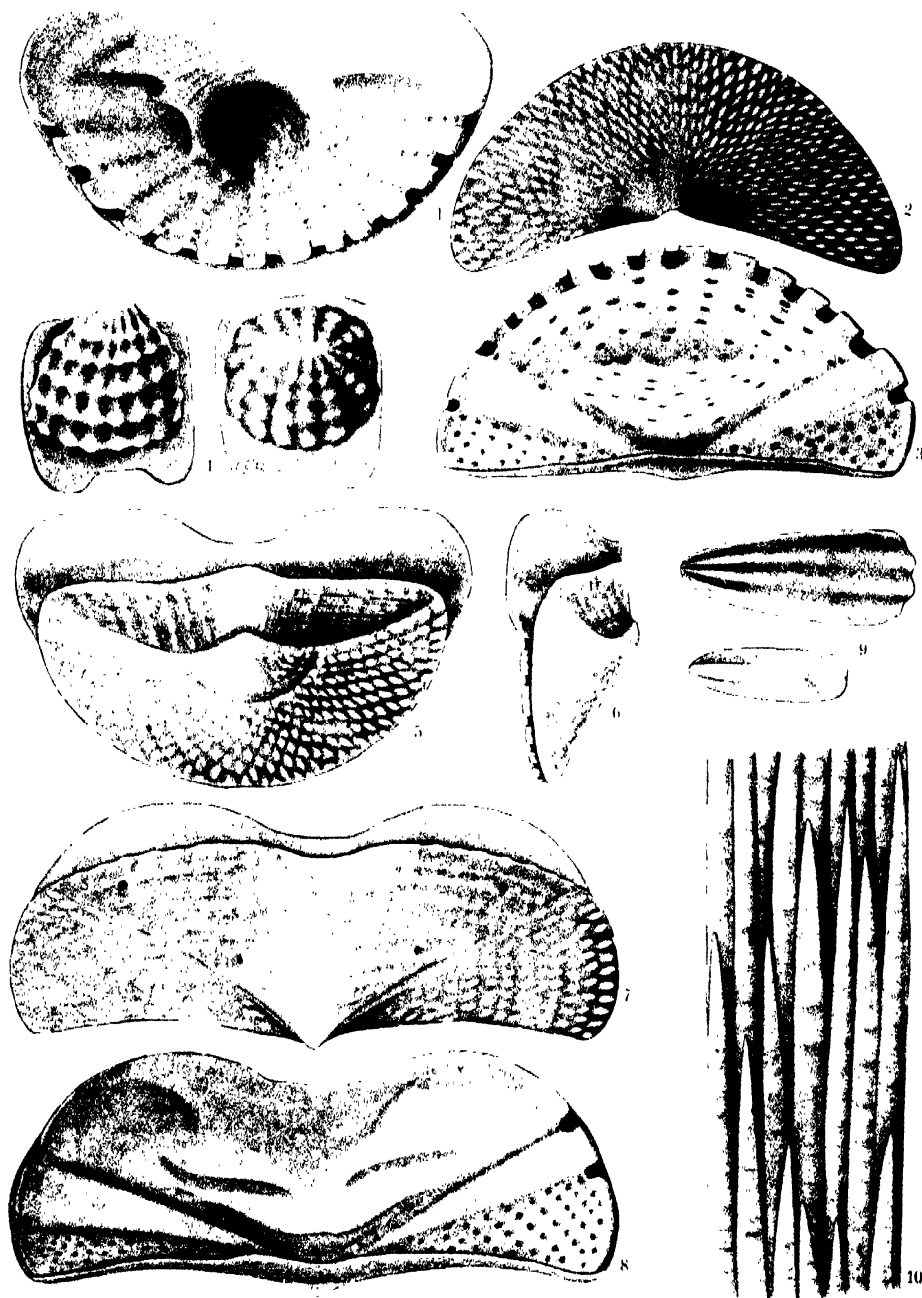
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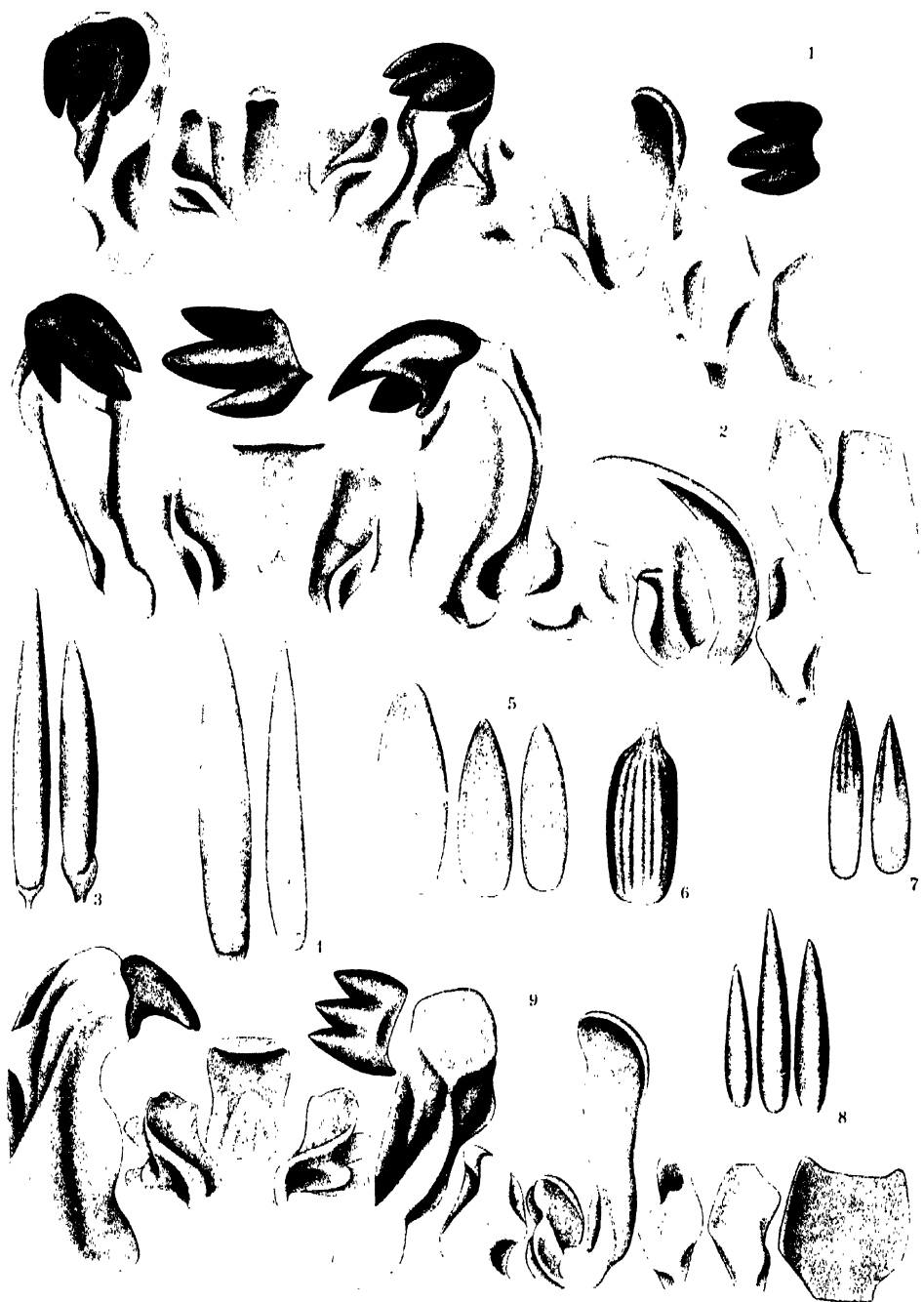




1. TAKI: Chitons of Mutsu Bay.

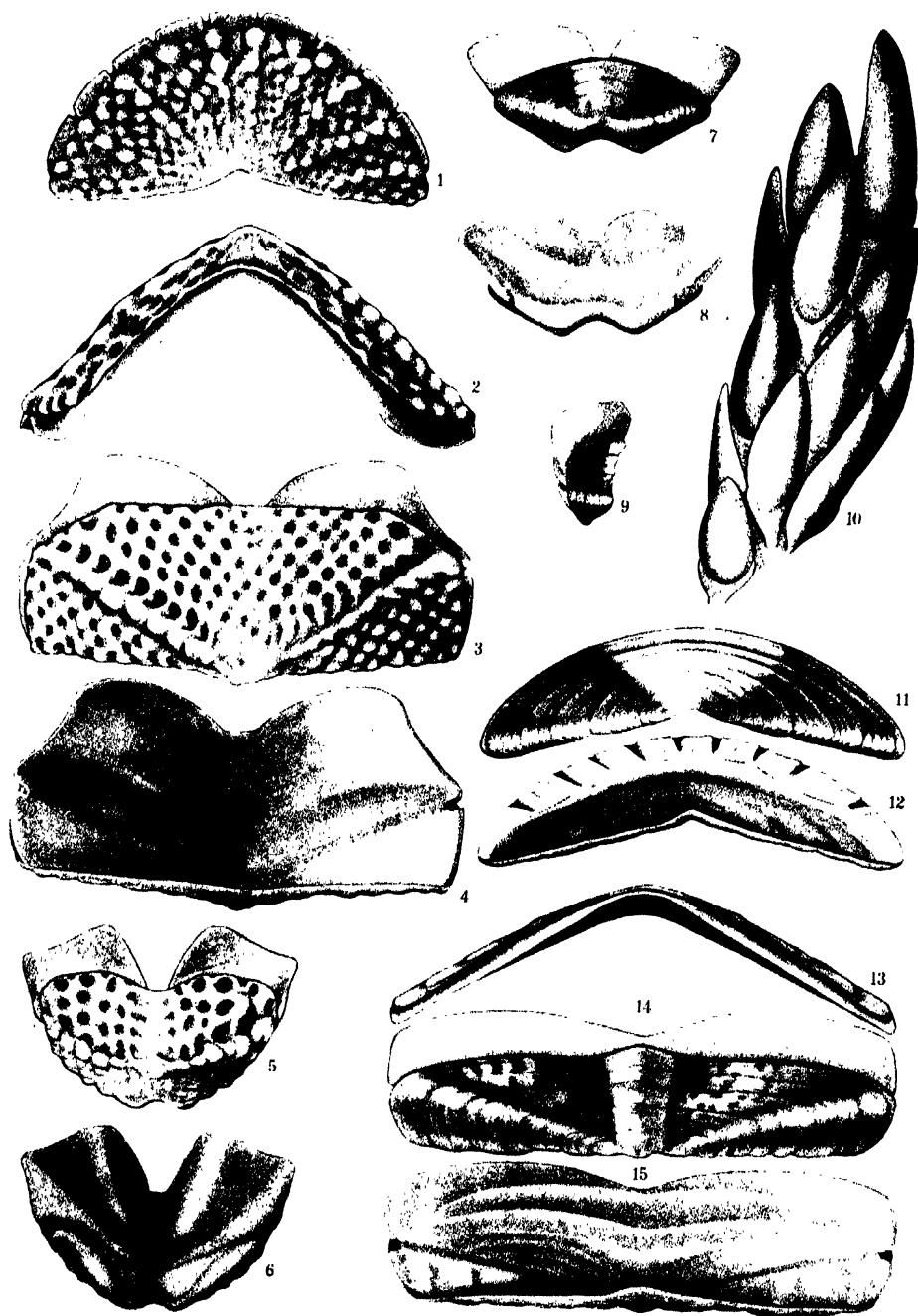






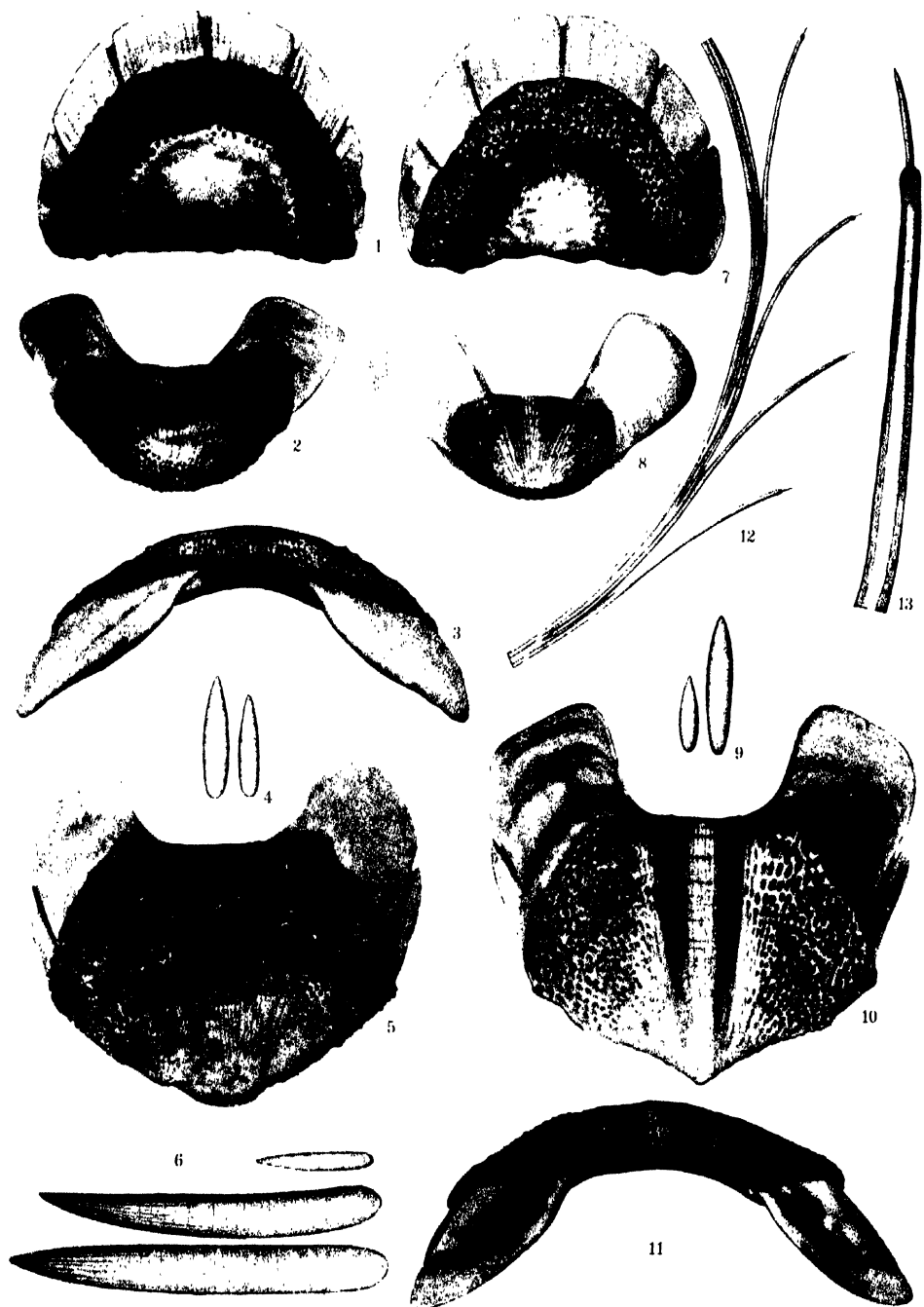
1. TAKI: Chitons of Mutsu Bay.





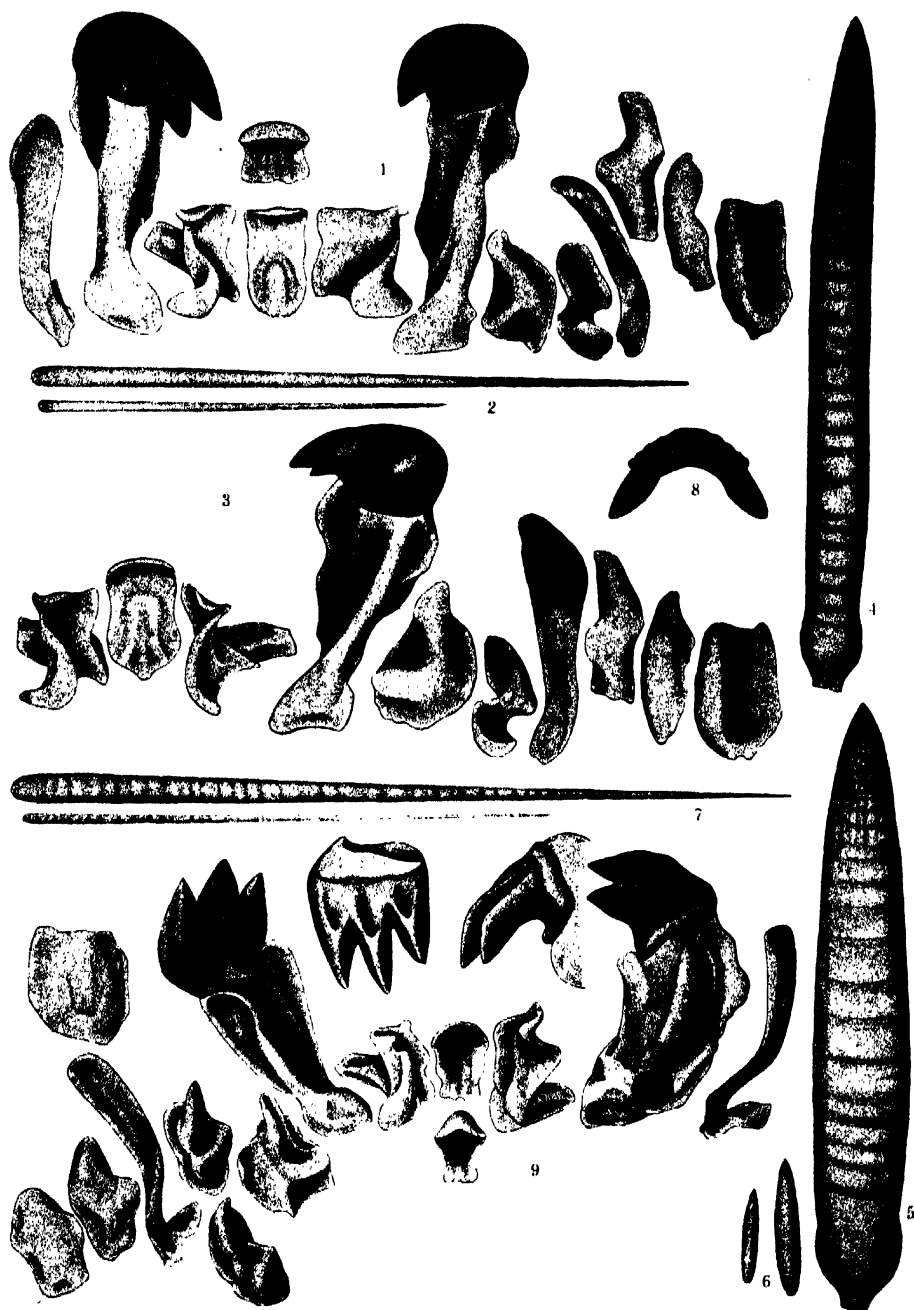
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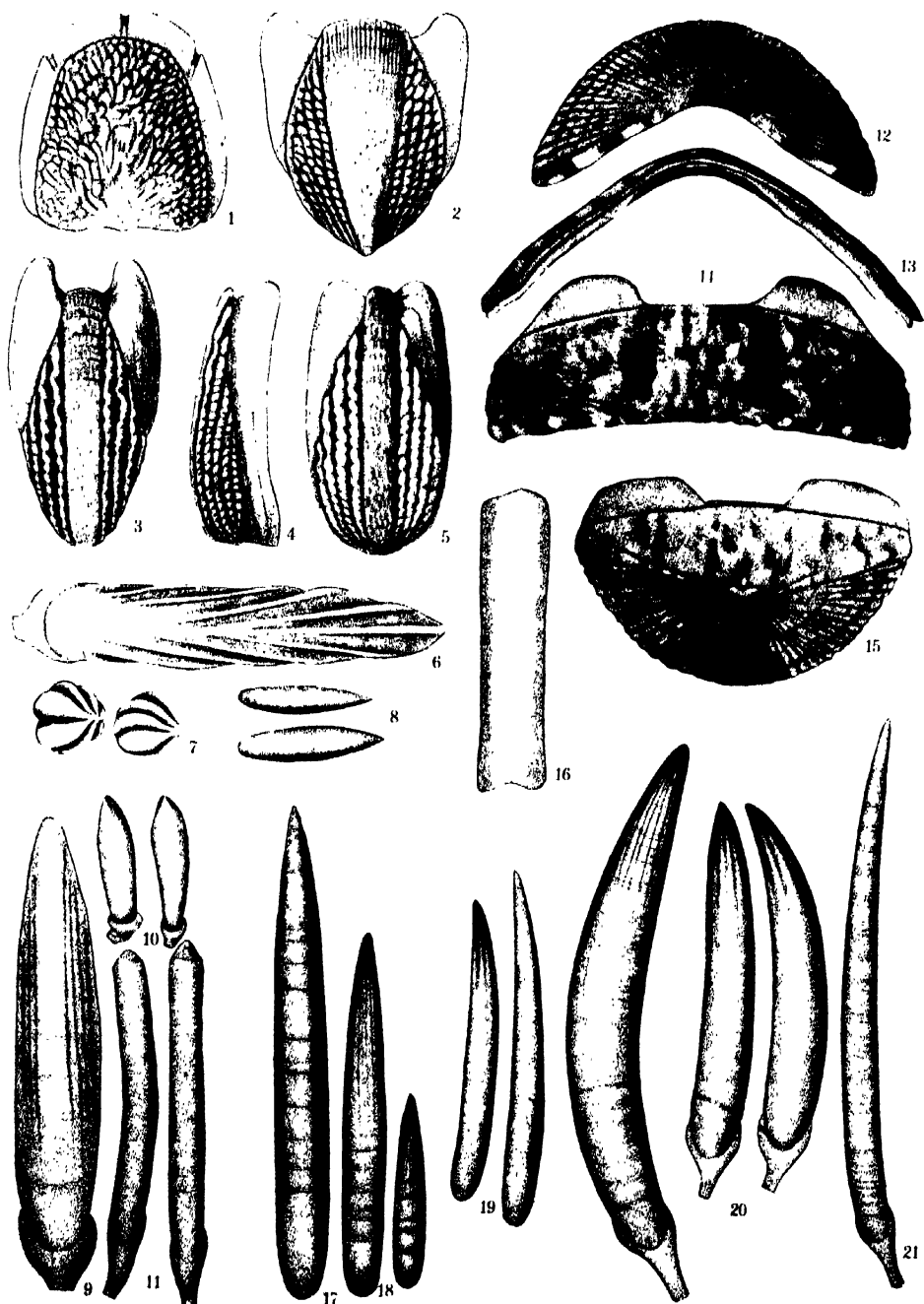




I. TAKI: Chitons of Mutsu Bay.

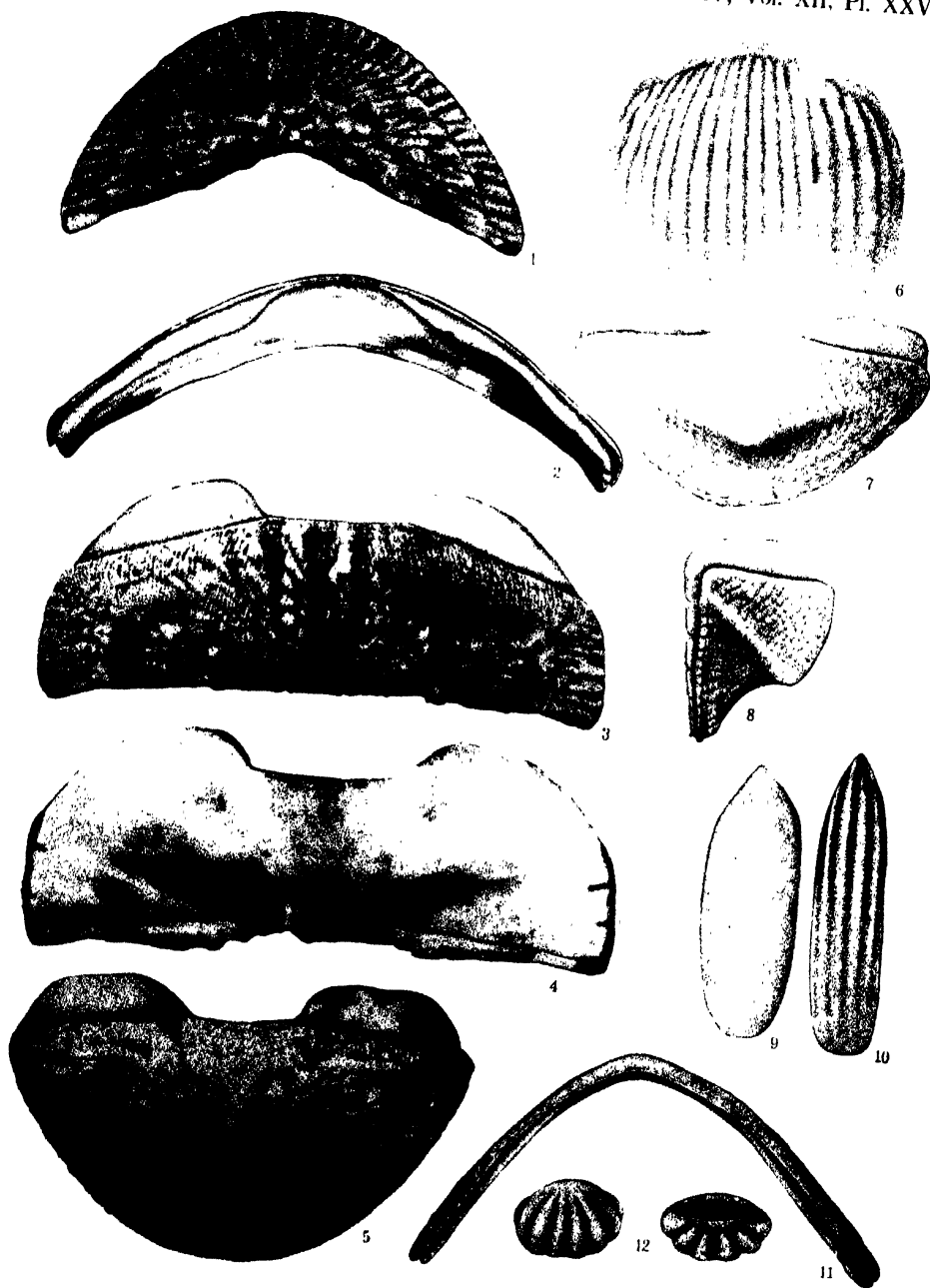






I. TAKI: Chitons of Mutsu Bay.





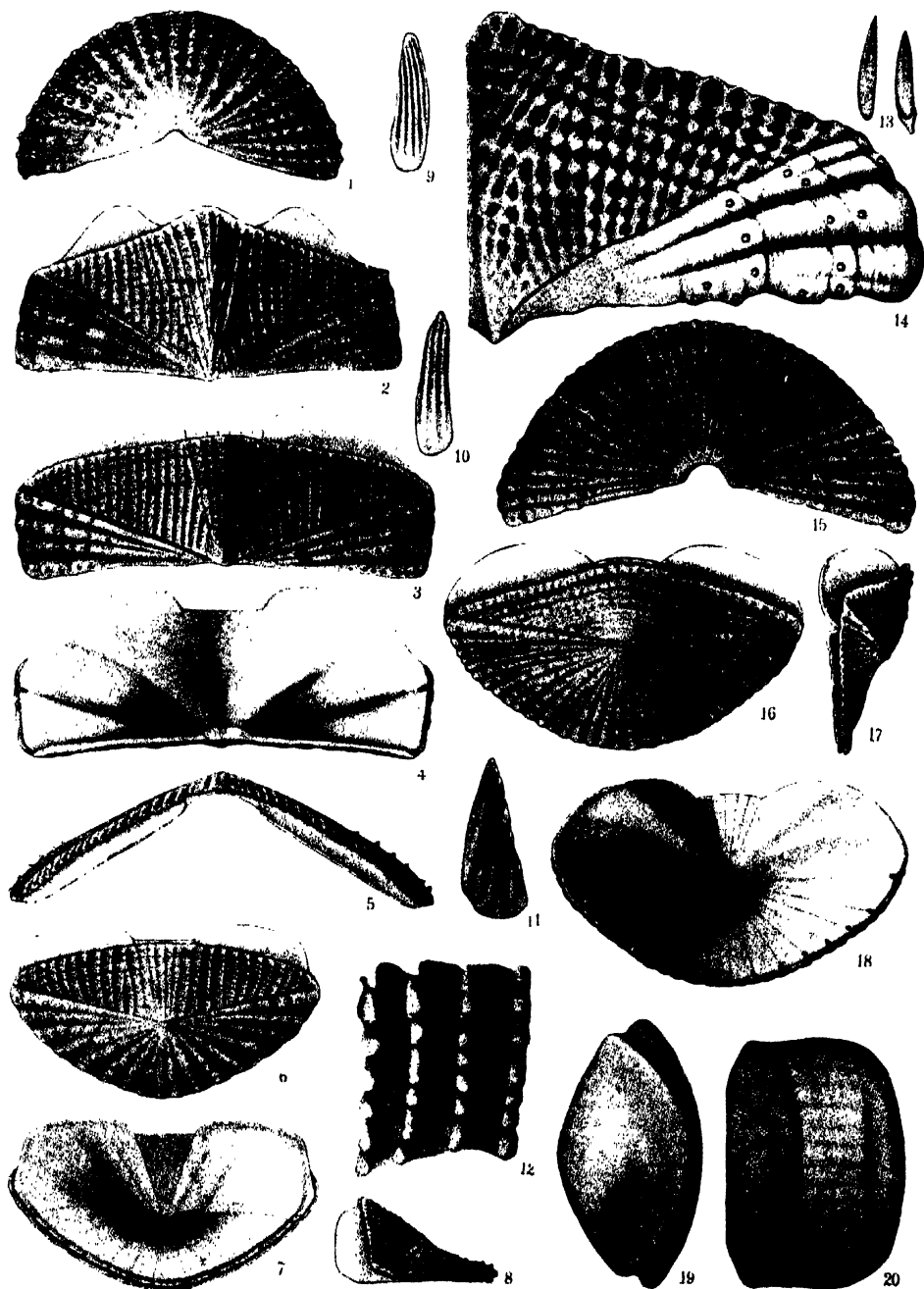
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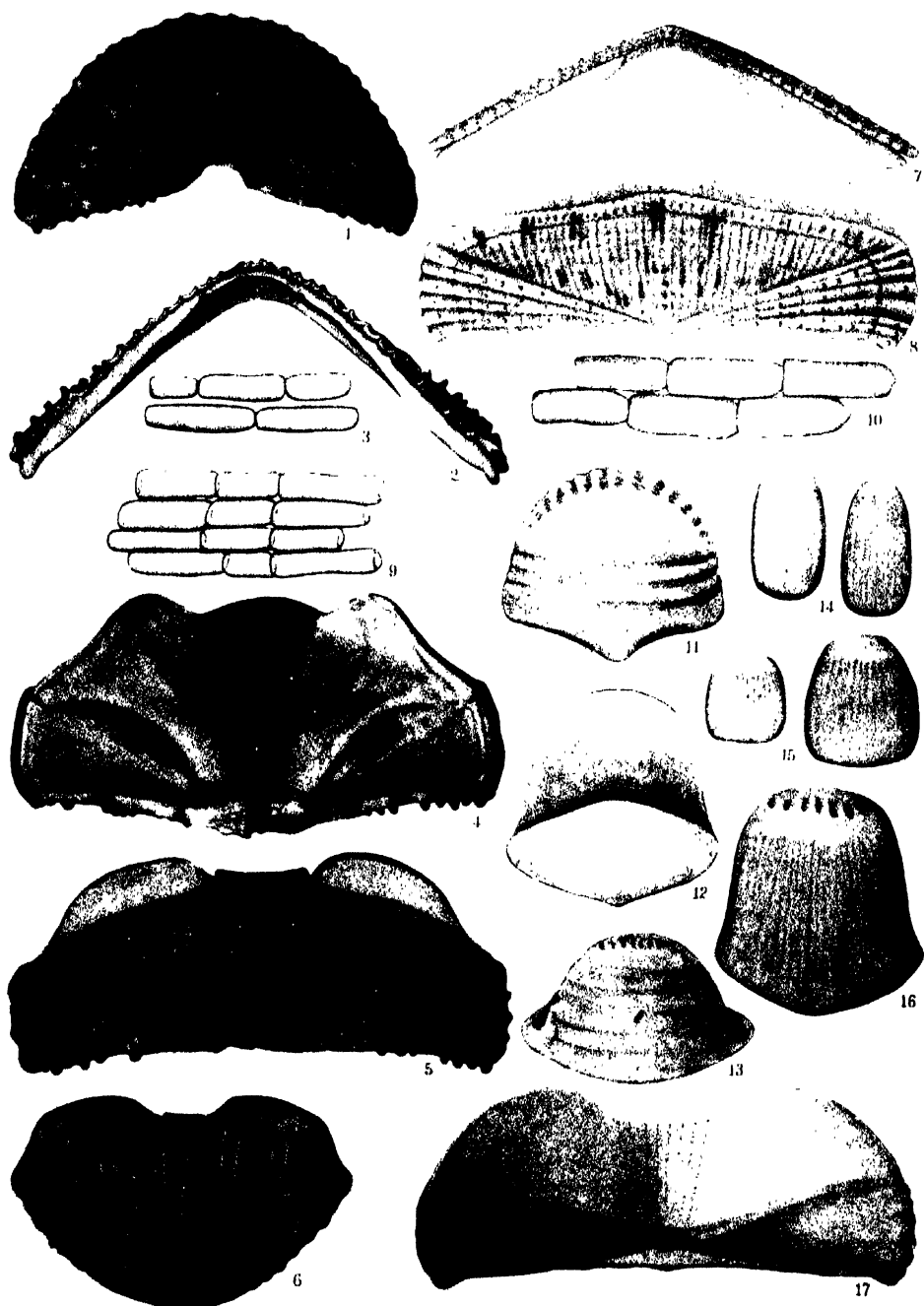




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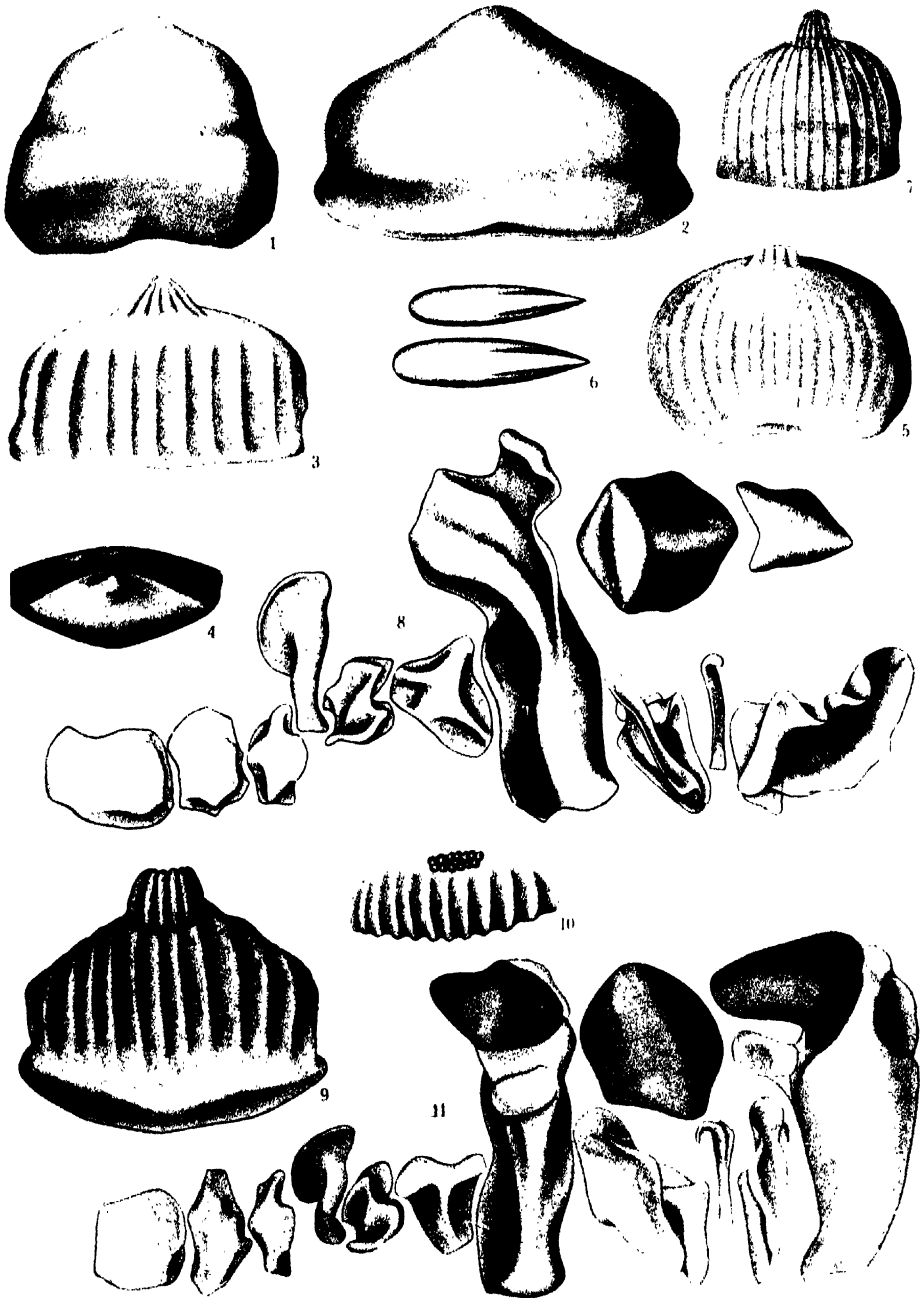
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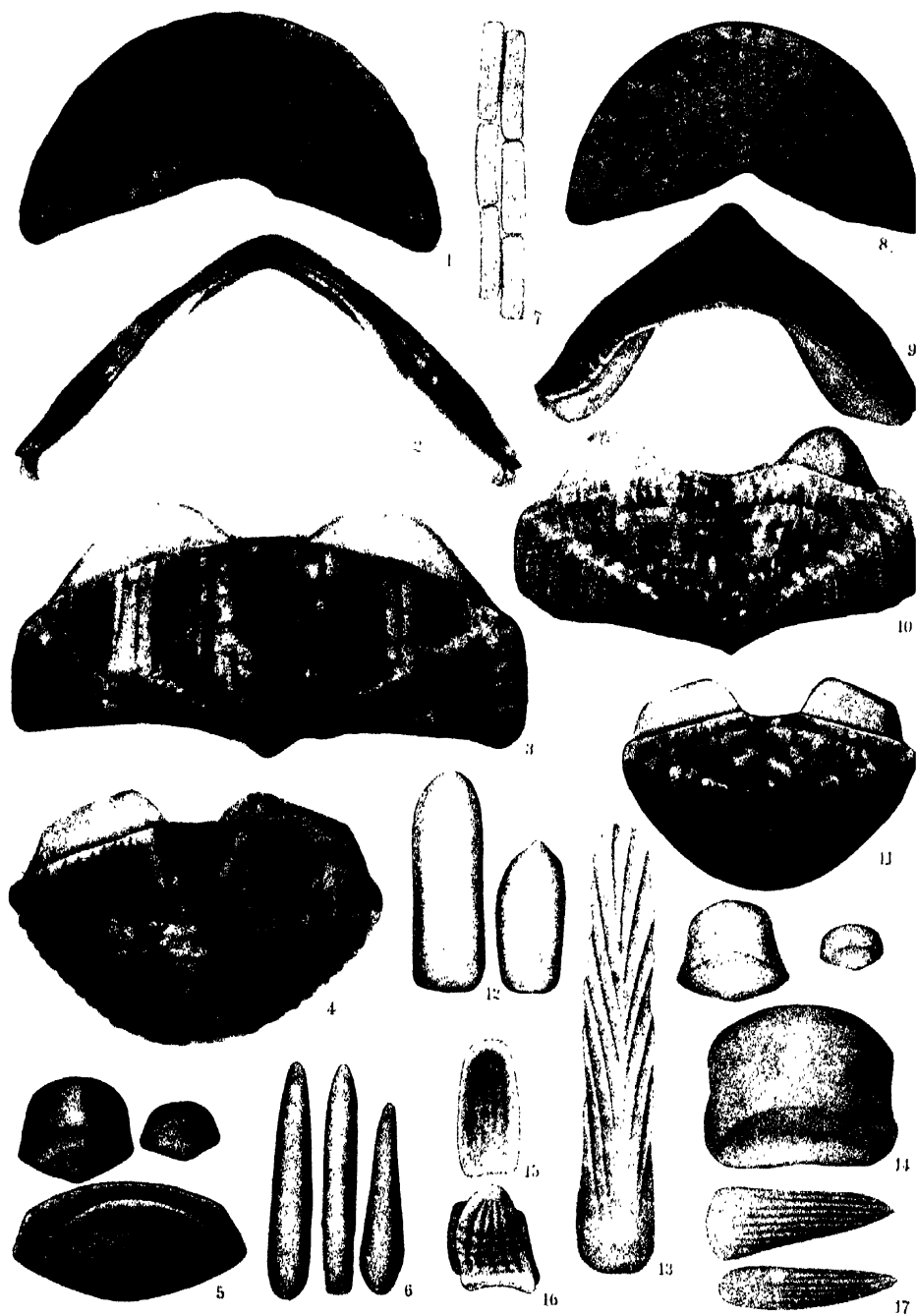
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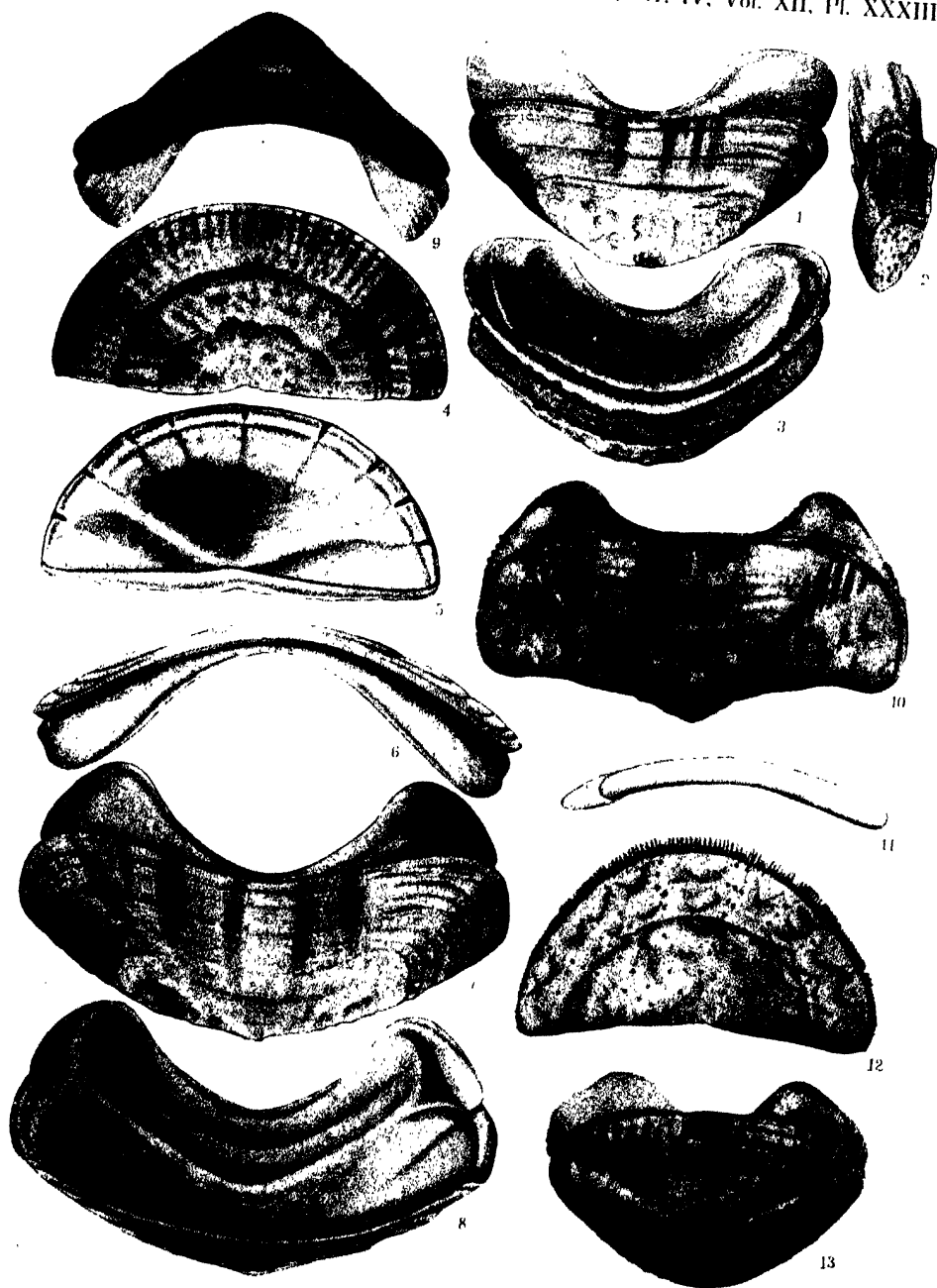




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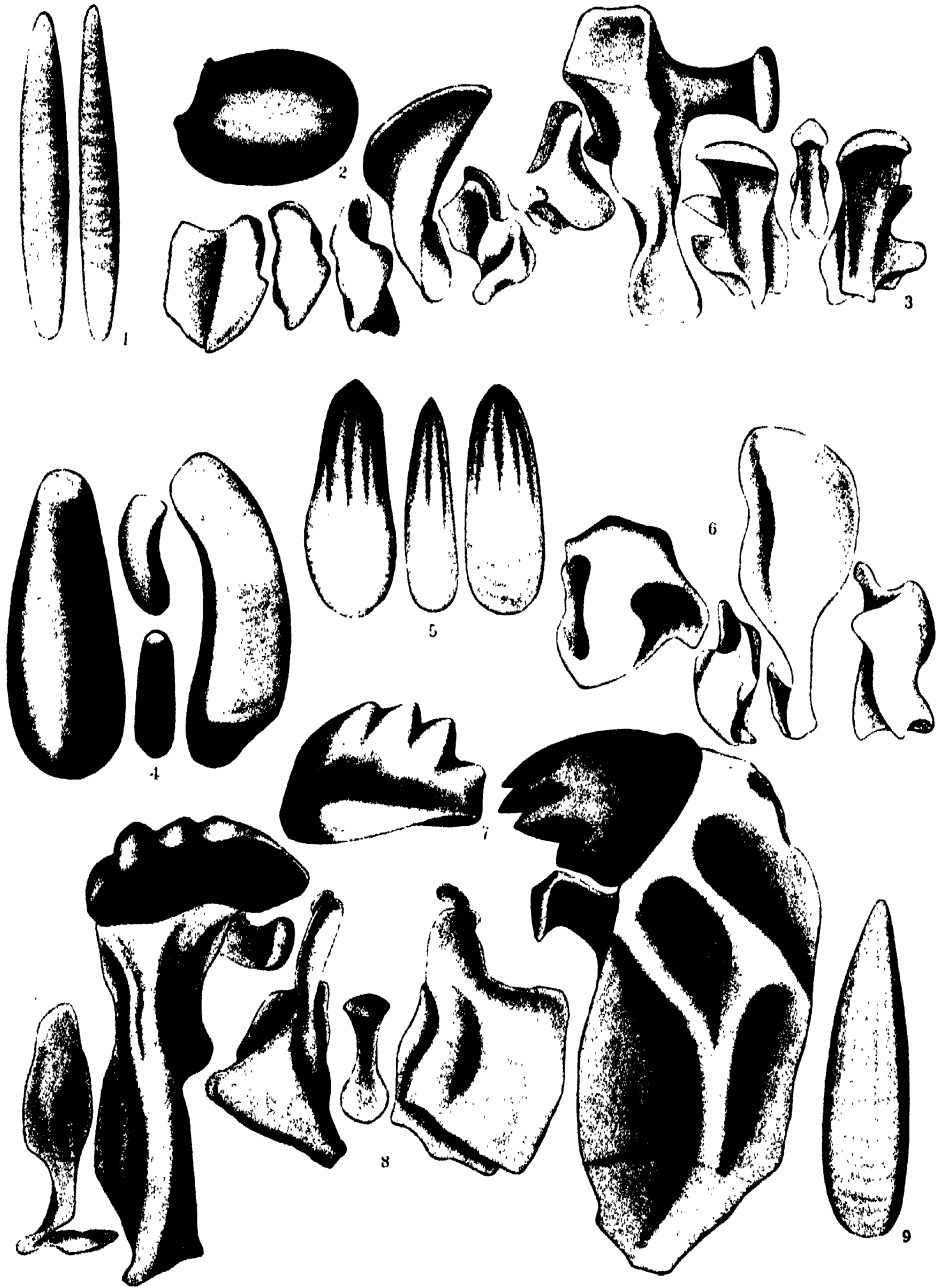






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# SOME NOTES ON *SPHAERIUM JAPONICUM BIWAENSE* MORI, A FRESHWATER BIVALVE

## V. PRODISSOCONCH-LARVA

By

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(With Plates XXXV-XXXVIII and sixty text-figures)

(Received September 10, 1937)

In the Sphaeriidae, the larva is reared in the marsupial sac of the inner gill of the mother-bivalve, through all the stages of development. The present writer has observed the early stages of such a viviparous development in *Sphaerium japonicum*, viz. those of the cleavage, of the gastrula, and of the fetal larva (OKADA 1935 c & 1936). In the further progress of development, the fetal larva grows directly into the earliest form of the bivalve without undergoing any apparent metamorphosis. The earliest bivalved larvae of the species under investigation however pass through the developmental stages within the marsupium, until delivery from the maternal body occurs. Thus, the developing larva of this bivalved form, after the coming into existence of fetal larva and before delivery, is called, in the present paper, the prodissoconch-larva.

In this paper, the present writer gives an outline of his morphological observations of the development of the 'prodissoconch-larva' of the *Sphaerium japonicum biwaense*.

Further, he is much indebted to Prof. Dr. E. NOMURA for his kind suggestions and criticism during the course of this study.

### MATERIAL AND METHOD

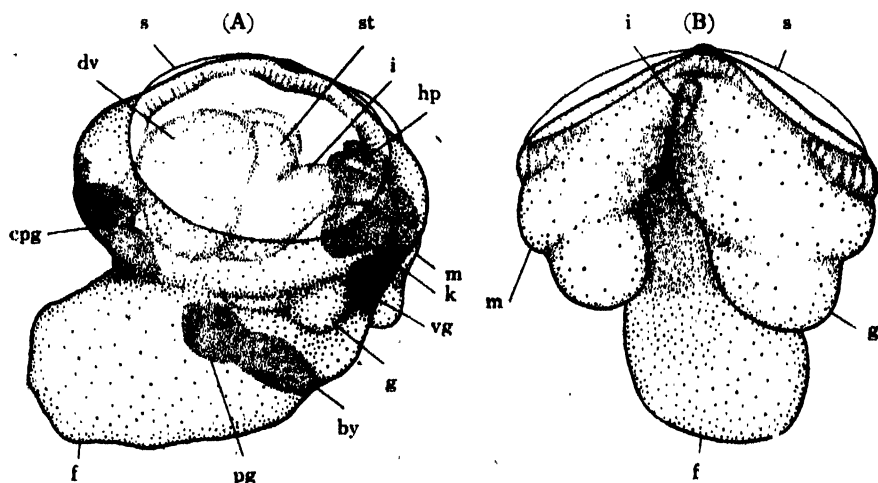
The material used in the present study was collected in April and June, 1936, from the drainage ditch of Iwanuma-Machi, a town south of Sendai. The specimens were ascertained to be those of *Sphaerium japonicum biwaense* MORI, according to the identification, most kindly made by Mr. SYUICHI MORI.

Investigations were carried out of both live and fixed specimens of the larva, the live ones being obtained by dissection from the inner gills of the mother-bivalve. Various methods of fixation and staining were tried,

and the fixation with Perényi's solution and the staining with boraxcarmine were found to be most suitable for the total preparation. For the purpose of observing the inner structure, the inner gills involving the larvae were removed from the specimens, which were killed with Zenker's solution, without glacial acetic acid, the embryonic shell being then decalcified in a 70% alcohol solution, containing 1% of acetic acid. These fixed specimens were sectioned serially, 7–10  $\mu$  in thickness, by the paraffin method, or, 30  $\mu$  in thickness, by the celloidin method, and then stained with Delafield's haematoxylin, or by Mallory's connective tissue staining method.

#### CHANGES IN EXTERNAL SHAPE

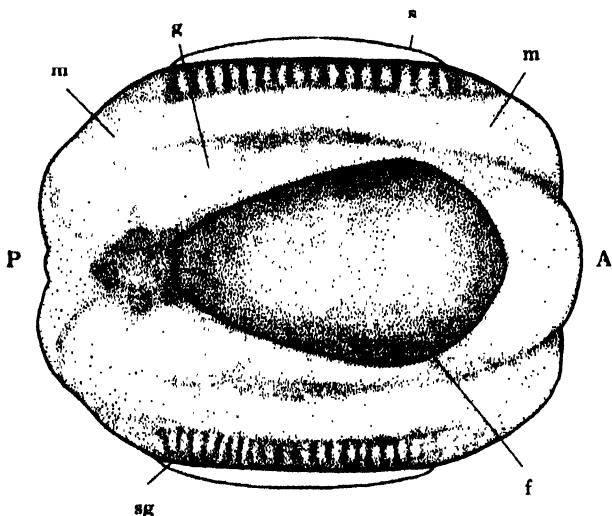
*Stage I.* The first stage of the prodissoconch-larva is continuous from the final stage of the fetal larva, without any notable metamorphosis. The external shape of this early prodissoconch-larva, measuring 0.25–0.3 mm. in body length (antero-posterior dimension), has the mushroom-like outline of the later fetal larva (Pl. XXXV, Fig. 1). In this stage, two remarkable characteristics are seen in connection with the body. One is the formation of a small, but distinct, prodissoconch on the dorsal side of the body, and the other is the appearance of the rudiments of paired mantles and gills in its ventral region (Text-fig. 1).



Text-fig. 1. Prodossoconch-larva in Stage I, about 0.25 mm. long.  $\times 200$ . (A) left side view, (B) posterior view. *by* byssal gland, *cp* cerebro-pleural ganglion, *dv* digestive diverticulum, *f* foot, *g* gill, *hp* heart and pericardium, *i* intestine, *k* kidney, *m* mantle, *pg* pedal ganglion, *s* prodossoconch, *st* stomach, *vg* visceral ganglion.

**Stage II.** In the course of its development, the larva increases in size and enters the second stage, when it measures 0.3–0.5 mm. in length. The external shape gradually resembles that of the adult in appearance, owing to the rapid extension of the mantle and shell. In this second stage, each extending portion of mantle covers the gill, the visceral sac, and the proximal portion of the foot (Pl. XXXV, Fig. 2), and also, when viewed from the ventral side, each gill rudiment gradually forms a little undulatory ridge inside the rudimentary mantle (Text-fig. 2).

**Stage III.** When the larva has grown to a length of over 0.5 mm., it enters the third or final stage, the adult outline being assumed. In this stage, the prodissoconch, the visceral sac, the mantle, the gill and the foot are clearly observable (Text-fig. 3). After this stage there is no further change in the form of the larva, which now develops into the bivalve in its earliest stage (Pl. XXXV, Figs. 3 & 4).



Text-fig. 2. Prodissoconch-larva in Stage II, about 0.35 mm. long, viewed from ventral side.  $\times 200$ . A anterior, P posterior; f foot, g gill, m mantle, s prodissoconch, sg margin of shell gland.

Some of the larvae, measuring 1–2.5 mm. in length, are readily liberated from the marsupial sac, but remain, for a while, in the inner branchial chamber of the mother-bivalve (OKADA 1935 b). Except that the byssal gland in the foot is now fully developed and functional, the external features of these larvae are little distinguishable from those of the young mussels already born (Pl. XXXV, Figs. 5–7). The byssal gland, functional at this stage, secretes a byssal thread attached to the walls of the inner branchial chamber. This gland in the present writer's species is a vestigial organ, and the delivery of the young mussels from the mother seems to be caused by the atrophy of the byssal thread. The size of the young, when delivered, differs in different individuals, and measures about 1.5–

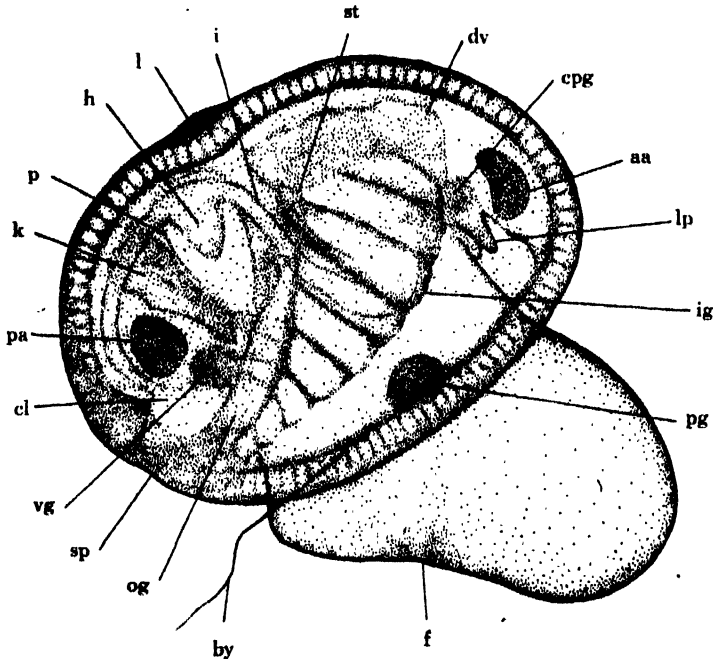


2.5 mm. in length.

## OUTLINE OF ORGANIZATION

### 1. SHELL GLAND AND PRODISSOCONCH

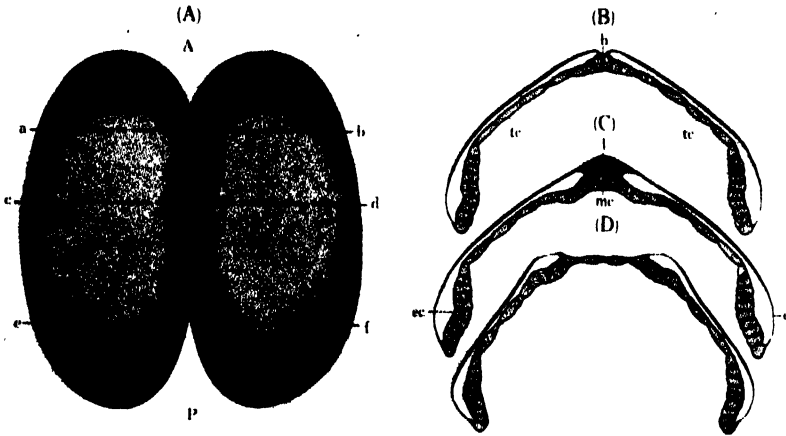
As already stated, the shell-epidermis, originating in the final stage of the fetal larva, develops directly into a prodissoconch, owing to a very active and more rapid extension of the shell gland in the lateral area



Text-fig. 3. Prodissoconch-larva in Stage III, about 0.7 mm. long, viewed from right side.  $\times 100$ . *aa* anterior adductor muscle, *by* byssus, *cl* cloacal chamber, *cpg* cerebro-pleural ganglion, *dv* digestive diverticulum, *f* foot, *h* heart, *i* intestine, *ig* inner gill, *k* kidney, *l* ligament of shell, *lp* labial palp, *og* outer gill, *p* pericardium, *pa* posterior adductor muscle, *pg* pedal ganglion, *sp* siphon, *st* stomach, *vg* visceral ganglion.

than in the median. The structure of the shell gland in the first and second stages of the prodissoconch-larva is shown in the figures in Plates XXXVI-XXXVIII, i. e. its dorsal and marginal parts consist of columnar cells, while its lateral areas become thinner in the course of its growth. Actually, the shell-epidermis is secreted by the columnar cells on the

marginal borders (Text-fig. 4). Thus, the limits of the shell gland coincide with those of the shell.



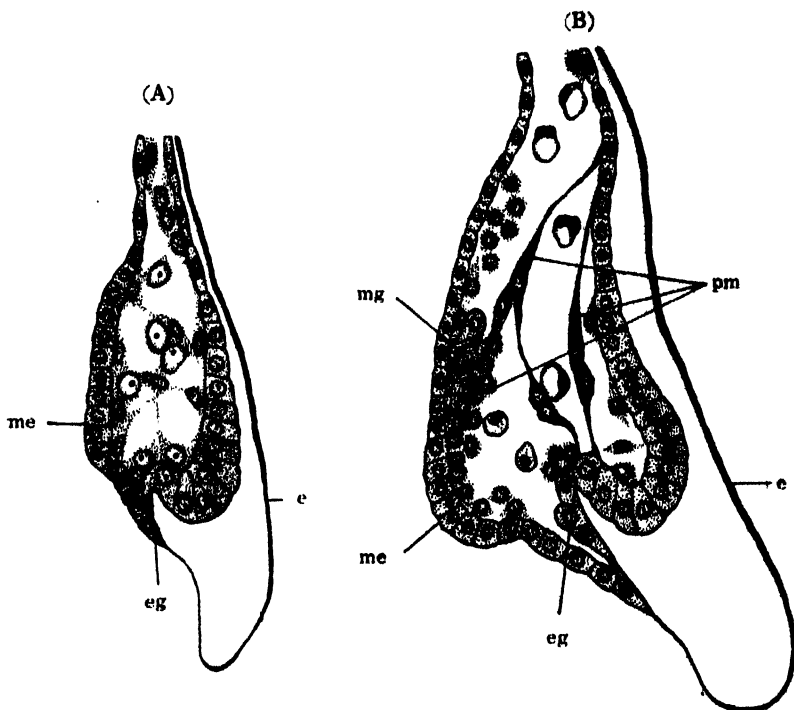
Text-fig. 4. Schematic representation of prodissoconch and shell gland of prodissoconch-larva in Stage II, about 0.4 mm. long.  $\times 150$ . (A) dorsal view of prodissoconch, (B) transverse section through *ab* in (A), (C) transverse section through *cd* in (A), (D) transverse section through *ef* in (A). *A* anterior, *P* posterior; *e* epidermis of shell, *ec* marginal columnar cells of shell gland, *h* hinge line, *l* ligament, *mc* dorsal columnar cells of shell gland, *tc* thinner cells of shell gland.

These marginal, columnar cells of the shell gland form the rudiments of the epidermal gland, and cuticular secretion from these cells is the origin of the epidermis of the prodissoconch. When the larva reaches the third stage, the epidermal gland lies near the outer margin of the mantle, and shows an involuted depression at the distal margin of the shell gland. The prodissoconch extends over the whole outer surface of the mantle, and the involuted epidermal gland deepens into a slit-like cleft, which represents the definitive differentiation of the gland at the outer margin of the mantle (Text-fig. 5). Thus the newly grown shell-epidermis is formed by secretion from this involuted cleft.

The ligament originates in the second stage of the larva. The right and left halves of the epidermis are continuous on the median, dorsal side of the shell gland. In this portion, the accumulation of secretion is observed between the shell-epidermis and the dorsal, columnar cells of the shell gland (Text-figs. 4 & 6, & Pl. XXXVIII, Fig. 11 c). This accumulated mass of secretion indicates the origin of the ligament, and is probably secreted by the dorsal, columnar cells of the shell gland, being

apparently composed of a substance, which fairly resembles that of the epidermis. The formation of the ligament indicates the definite position of the future hinge line (Text-fig. 4).

In the third stage of the larva, the columnar cells at the median, dorsal side of the shell gland increase in height, propagate further as the

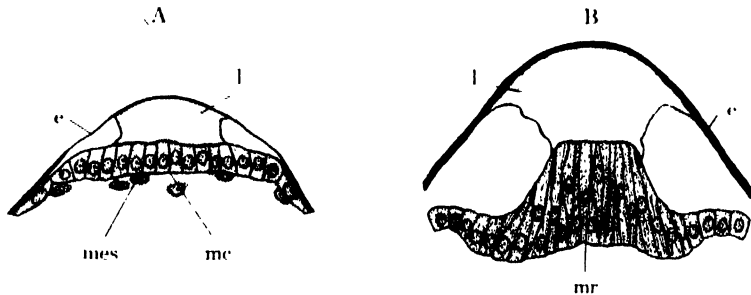


Text-fig. 5. Transverse sections of prodissococonch-larva in Stage III, showing epidermal gland and mantle edge.  $\times 400$ . (A) mantle edge of larva, about 0.5 mm. long, (B) mantle edge of larva, about 1.5 mm. long. *e* epidermis of shell, *eg* epidermal gland, *me* mantle edge. *mg* mucous-secreting glandular cells, *pm* pallial muscle fibres.

consequence of the addition of their secretion to the initiative ligament, and form a short, median ridge at the dorsal side of the shell gland (Text-figs. 6 & 8).

It is difficult to investigate with any accuracy the mechanism of the deposition of carbonates in the shell. At any rate, it is true that an interspace soon appears between the epidermis and the surface of the shell gland, and seems to contain a specialized liquid, which is produced by some unknown mechanism over the entire area of the shell gland, being

probably caused by a certain physiological activity. In a live specimen, about 1 mm. in length, the internal organization can be seen through the shell, owing to its transparency (Pl. XXXV, Figs. 3 & 5); while, in a fixed specimen at the same stage, it cannot be seen, because of the intransparency due to the coagulation of the organic matter contained in the shell (Pl. XXXV, Figs. 4 & 6).



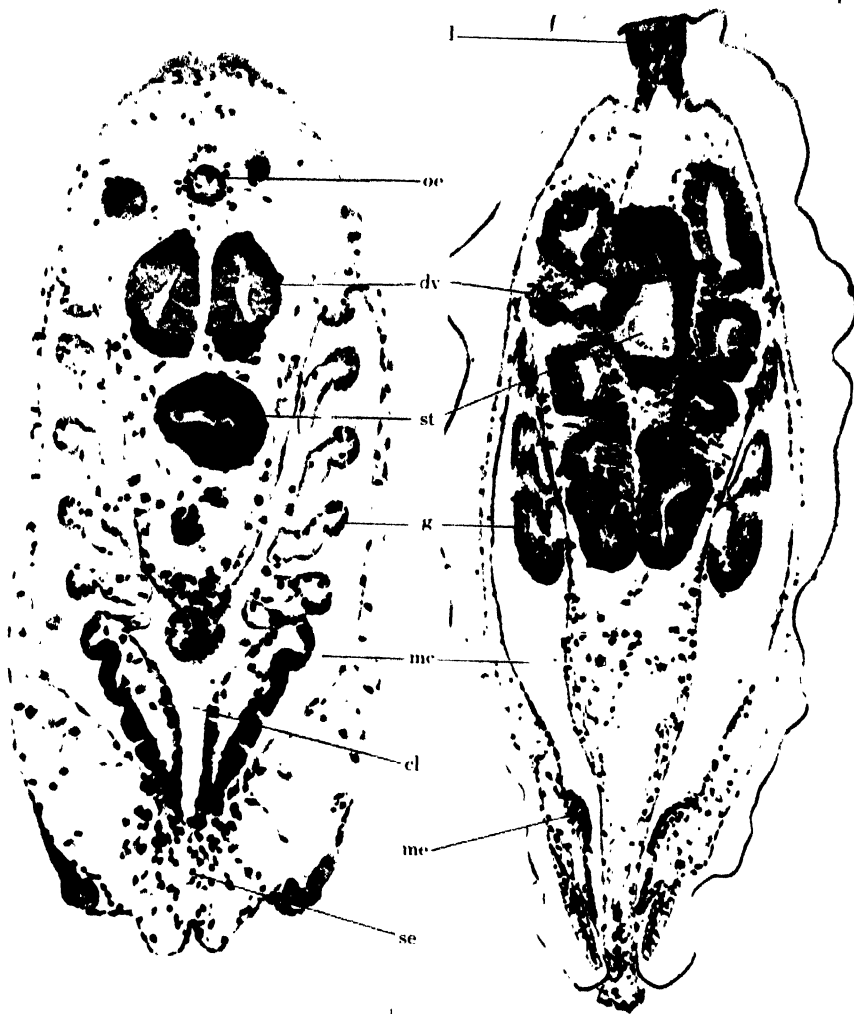
Text-fig. 6. Transverse sections of prodissoconch-larva, showing ligament and related structures.  $\times 400$ . A ligament of larva, about 0.4 mm. long, (B ligament of larva, about 0.8 mm. long. *e* epidermis of shell, *l* ligament of shell, *mc* dorsal columnar cells of shell gland, *mes* mesenchyme, *mr* dorsal ridge of median shell gland.

## 2. MANTLE, GILL, FOOT, AND VISCERAL SAC

*The Mantle.* In the first stage of the prodissoconch-larva, in the region of the postero-ventral body wall, a pair of remarkable tubercles are found immediately behind the foot. These tubercles are the rudiments of the mantle folds (Text-fig. 1). In the progress of development, the rudimentary mantle fold, on either side of the body, extends anteriorly, and forms a rudimentary mantle cavity on its inner side.

In the second stage, each mantle fold begins to cover laterally the visceral sac, the rudimentary gill, and the proximal region of the foot, in connection with the rapid extension of the shell gland and with the progress of a bilateral compression of the body, which appears at the junction between the foot and the visceral sac (Text-fig. 2). The structure of the mantle at this stage is only a large folding of the lateral body wall. The epithelium of the mantle consists of flattened cells on the inner surface, of cubical cells in the distal area, and of the cells of the shell gland on the outer surface (Pls. XXXVII & XXXVIII). Mesenchymal elements are seen in the interior of the mantle, especially near its distal margin.

When the larva enters the third stage, the differentiation of the so-called mantle edge becomes apparent. The distal margin of the mantle, con-



Text-fig. 7. Horizontal section through body of prodissococonch-larva in Stage III, about 0.9 mm. in length, showing gill, stomach, and digestive diverticula. Photo. from specimen stained by Mallory's connective tissue staining method.  $\times 150$ .

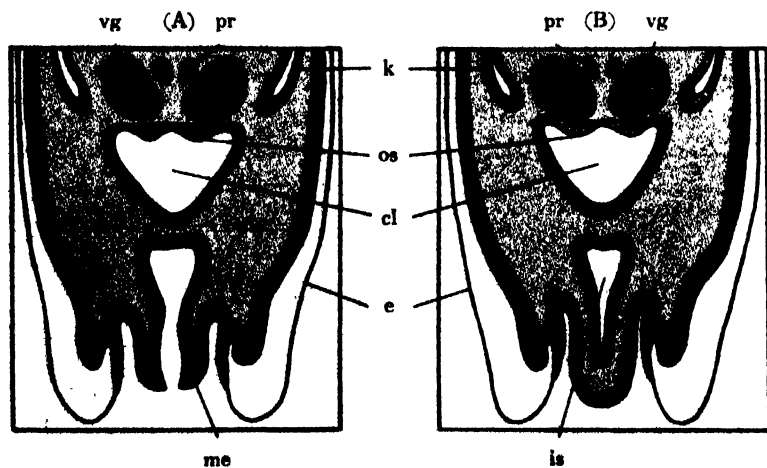
Text-fig. 8. Transverse section through body of prodissococonch-larva in Stage III, about 1.2 mm. in length, showing ligament of shell, digestive diverticula, and mantle edges. Photo. from specimen stained by Mallory's connective tissue staining method.  $\times 150$ .

*cl* cloacal chamber, *dv* digestive diverticulum, *g* gill, *l* ligament of shell, *mc* mantle cavity, *me* mantle edge, *oe* oesophagus, *se* united septum of mantle, *st* stomach.

sisting of cubical cells, forms an elevation towards the mantle cavity, and, internally, becomes attached by connective tissue cells, or pallial myocytes. At the same time, the inner layer of the mantle becomes increasingly flattened and forms a thinner epithelium, together with the flattened area of the shell gland on the outer surface of the mantle.

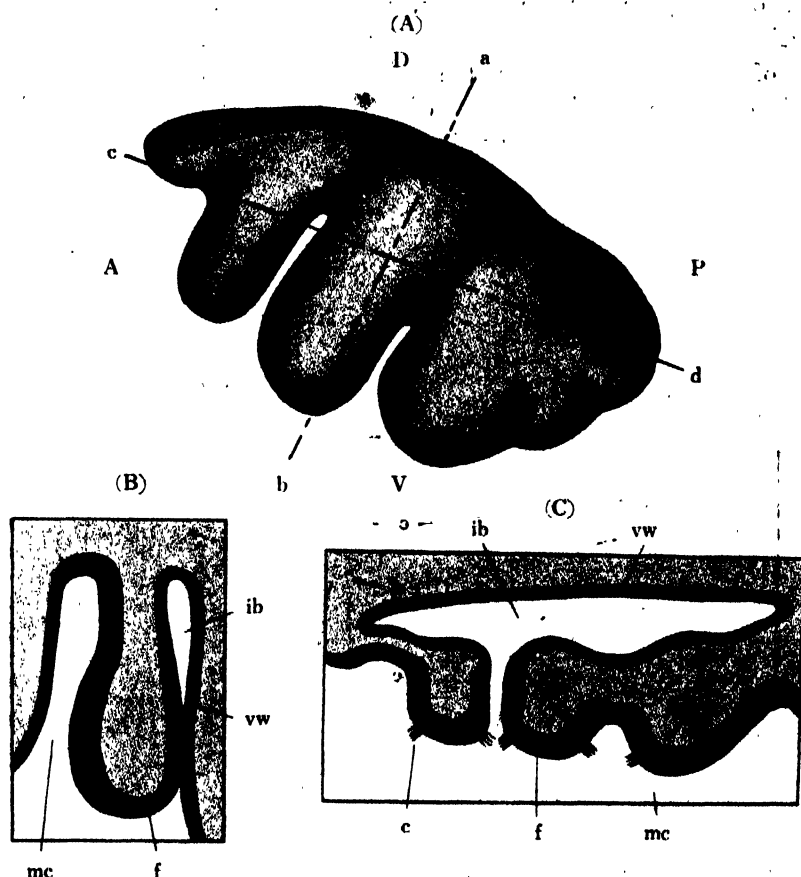
At the end of the third stage, the siphons are formed at the postero-ventral area of the mantle. When the larva measures about 0.7 mm. in length, both mantle edges unite with each other at the point, where the posterior end of each gill is attached to the corresponding mantle, and form, obliquely, a median septum, which separates the dorso-posterior cloacal chamber from the ventro-anterior mantle cavity. With the development of the cloacal chamber, the exhalant siphon is first formed by the mantle edges on the dorsal side of this septum, and then the inhalant siphon is formed by another union of the mantle edges on the ventral side of it (Text-figs. 7 & 9).

*The Gill.* In the first stage of the prodissococonch-larva, immediately after the formation of the rudimentary mantle, already described, a tubercle is observed on the inner surface of each mantle fold. This swelling inside the mantle is the rudiment of a gill (Pl. XXXVI, Figs. 8 c & 9).



Text-fig. 9. Schematic representation of prodissococonch-larva in Stage III, illustrating formation of siphon.  $\times 150$ . (A) transverse section through cloacal chamber and mantle edge forming inhalant siphon, (B) transverse section through exhalant and inhalant siphons. *cl* cloacal chamber or base of exhalant siphon, *e* epidermis of shell, *is* inhalant siphon, *k* kidney, *me* mantle edge forming inhalant siphon, *os* osphradium, *pr* posterior retractor muscle of foot, *vg* visceral ganglion.

Its surface is a single layer constituted of cubical elements, and the several invading mesenchymes fill its interior. Each rudimentary gill is thrust out postero-ventrally and, when viewed laterally, it is a triangular ridge protruding under the mantle fold (Text-fig. 1). Meanwhile, the rudimentary gill begins to differentiate into filaments. On the outer surface of the rudimentary gill, a few undulatory foldings appear obliquely when viewed laterally. These folds are the initiative gill filaments, and the gill comes to be composed of a set of filaments, which constitute the earliest gill lamella.



Text-fig. 10. (A) reconstructive schema of rudimentary gill of prodissococonch-larva in Stage II, about 0.4 mm. long, and diagram illustrating its structure in transverse section (B) through *ab*, and in horizontal section (C) through *cd* in (A).  $\times 400$ . A anterior, D dorsal, P posterior, V ventral; c cilia of gill filament, ib inner branchial chamber, m mantle, mc mantle cavity, vw wall of visceral sac.

At the beginning of the second stage of the larva, each gill lamella extends anteriorly from the posterior end of the body to the level of the digestive diverticulum, and consists of three or four filaments (Text-fig. 2). Ciliated cells, also, appear at some places in each filament (Text-fig. 10). At the end of the second stage, the gill lamella becomes perfectly covered by the mantle owing to an active growth of the latter, although in the former, also, the number of filaments continues to increase, and, consequently, an extension of the lamella occurs (Pl. XXXV, Fig. 2). Henceforth, the differentiation of the gill advances to its definitive, filamental structure in the order from the anterior to the posterior side (Text-figs. 28-31).

In the third stage, the filamental structure of the gill seems to be almost complete, except at the insufficiently differentiated part near its posterior end (Text-figs. 3, 7 & 8). Simultaneously, a new tubercle of another gill comes to be observed on the posterior side of, and outside, each old gill. This old gill is the inner gill, and each newly formed one develops to the outer gill.

In the Sphaeriidae, the gill is the organ of respiration, and of the rearing of the young. Observation of the development of the gill, therefore, must be the most important and interesting part of the investigation. The actual results of this observation, however, cannot be definitely stated in this paper.

*The Foot.* As previously stated (OKADA 1936), the foot was distinct from the body in the final stage of the fetal larva. The pedal ganglia, the byssal gland, and many mesenchymal cells were found in this foot, which was a little protrusible, and had fine cilia on the surface of its distal portion.

In the first stage of the prodissoconch-larva, the formation of paired otocysts is observed at the anterior, proximal part of the foot (Pl. XXXVI, Fig. 8 a). Later than the second stage, the foot becomes more protrusible with the formation of definite muscles in its interior, and grows to be a muscular protrusion at the ventral side of the body, containing the pedal ganglia, the byssal gland, and the otocysts.

*The Visceral Sac.* At the beginning of the first stage of the prodissoconch-larva, the differentiation of the mantle and the gill from the body begins, and, henceforth, the visceral sac is distinct. When the larva reaches the second stage, the visceral sac thus proves to be the body proper, excluding the mantle, the gill, and the foot, and involves the main nervous system, the whole alimentary system, and many other organs, viz. the



heart, the pericardium, the kidney, the gonad, several muscles, etc. At the end of the second stage, the body wall on both sides of the mouth differentiates into labial palps. In the third stage, the paired rudiments of osphradia appear at the roof of the cloacal chamber.

Thus, all the organs appear at the definitive positions and develop into the adult organization, at the end of the last stage in the prodissococonch-larva.

## ORGANOGENESIS IN EACH SYSTEM

### 1. NERVOUS SYSTEM

*The Cerebral (Cerebro-pleural) Ganglia.* In the final stage of the fetal larva, on either side of the stomodaeum, the larval pleural ganglion was united with the larval cerebral ganglion, and the larval cerebro-pleural ganglion was formed there. Meanwhile, each larval cerebro-pleural ganglion became differentiated into a central mass of nerve fibres and peripheral

Text-figs. 11-18 are photographed from specimens stained by Mallory's connective tissue staining method.  $\times 300$ . *aa* anterior adductor muscles, *br* branchial lobe of visceral ganglion, *cc* cerebral commissure, *cg* cerebral part of cerebro-pleural ganglion, *cl* cloacal chamber, *cvc* cerebro-visceral connective, *dv* digestive diverticulum, *g* gill, *in* intestine, *k* kidney, *m* mouth, *mc* mantle cavity, *oe* oesophagus, *os* osphradium, *pc* pedal commissure, *pg* pedal ganglion, *plg* pleural part of cerebro-pleural ganglion, *pr* posterior retractor muscle of foot, *vc* visceral commissure, *vg* visceral ganglion.

Text-fig. 11. Horizontal section through prodissococonch-larva in Stage II, showing cerebral ganglia and cerebral commissure.

Text-fig. 12. Horizontal section through prodissococonch-larva in Stage II, showing united cerebral ganglia and pleural ganglia.

Text-fig. 13. Horizontal section through prodissococonch-larva in Stage II, showing pedal ganglia.

Text-fig. 14. Horizontal section through prodissococonch-larva in Stage II, showing visceral ganglia and their branchial lobes.

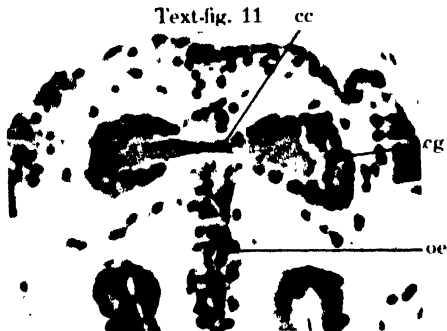
Text-fig. 15. Horizontal section through prodissococonch-larva in Stage III, showing cerebro-pleural ganglia, cerebral commissure, and anterior adductor muscle.

Text-fig. 16. Horizontal section through prodissococonch-larva in Stage III, showing united pedal ganglia.

Text-fig. 17. Horizontal section through prodissococonch-larva in Stage III, showing visceral ganglia and osphradia.

Text-fig. 18. Transverse section through prodissococonch-larva in Stage III, showing united visceral ganglia, visceral commissure, osphradia, posterior retractor muscles of foot, and cloacal chamber.

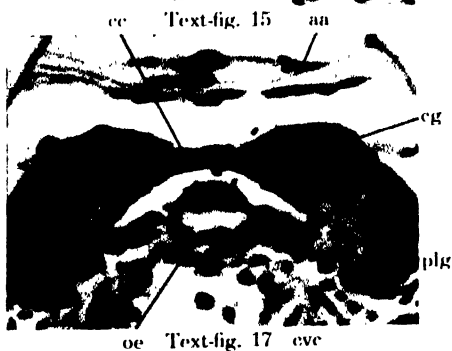
Text-fig. 11



Text-fig. 13



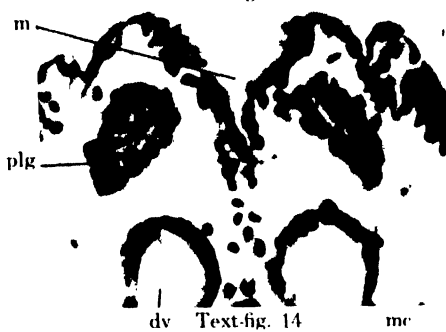
Text-fig. 15



Text-fig. 17



Text-fig. 12



Text-fig. 14



Text-fig. 16



Text-fig. 18



Text-fig. 19



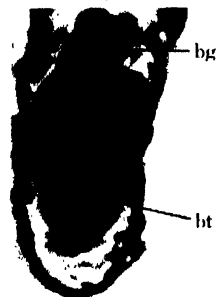
Text-fig. 20



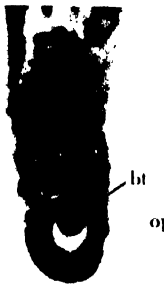
Text-fig. 25



Text-fig. 21



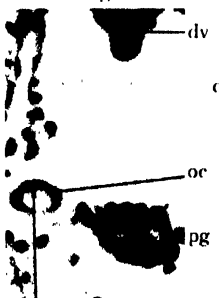
Text-fig. 22



Text-fig. 26



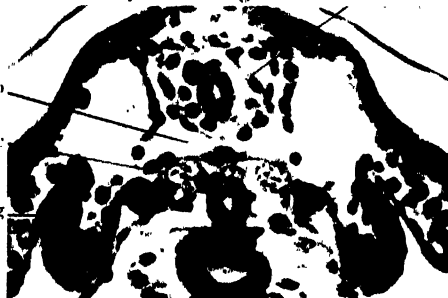
Text-fig. 23



Text-fig. 24



Text-fig. 28



Text-fig. 27



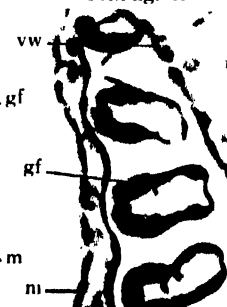
Text-fig. 31



Text-fig. 30



Text-fig. 29



layer, rich in nuclei. The paired cerebro-pleural ganglia were united by the pre-existing cerebral commissure, which passes over the anterior side of the stomodaeum.

In the first and second stages of the prodissoconch-larva, each cerebro-pleural ganglion is pear-shaped, with the cerebral part at the wider end and the pleural part at the narrower (Text-figs. 11 & 12). In the third

Text-figs. 19-25 and 28-31 are photographed from specimens stained following Mallory's connective tissue staining method, and Text-figs. 26 and 27 from specimens stained with Heidenhain's haematoxylin.  $\times 300$ . *ar* anterior retractor muscle of foot, *bg* byssus-secretory glandular cells, *bt* byssus, *by* byssal invagination, *dv* digestive diverticulum, *ef* epithelium of foot, *ep* epidermal gland, *f* foot, *g* gill, *gc* germ cells, *gf* gill filament, *gl* gill lamella, *h* heart, *in* intestine, *ip* inner labial palp, *lp* labial palp, *m* mantle, *mc* mantle cavity, *ms* mucous-secretory cells, *oc* otocyst, *ol* otolith, *op* outer labial palp, *p* pericardium, *pg* pedal ganglion, *plg* pleural part of cerebro-pleural ganglion, *vw* wall of visceral sac.

Text-fig. 19. Transverse section through prodissoconch-larva in Stage II, showing byssal invagination.

Text-fig. 20. Transverse section through prodissoconch-larva at beginning of Stage III, showing byssal gland.

Text-fig. 21. Transverse section through prodissoconch-larva at end of Stage III, showing fully developed byssal gland.

Text-fig. 22. Transverse section through prodissoconch-larva at end of Stage III, showing external orifice of fully developed byssal gland.

Text-fig. 23. Transverse section through prodissoconch-larva in Stage II, showing otocyst.

Text-fig. 24. Transverse section through prodissoconch-larva in Stage III, showing otocyst.

Text-fig. 25. Transverse section through prodissoconch-larva in Stage II, showing rudiment of labial palp and pleural part of cerebro-pleural ganglion.

Text-fig. 26. Transverse section through prodissoconch-larva at end of Stage III, showing labial palps, cerebro-pleural ganglia, and anterior retractor muscles of foot.

Text-fig. 27. Transverse section through prodissoconch-larva at end of Stage III, showing mucous-secretory cells in foot.

Text-fig. 28. Transverse section through prodissoconch-larva at beginning of Stage II, showing rudimentary gills, heart and pericardium, and primordial germ cells.

Text-fig. 29. Transverse section through prodissoconch-larva at beginning of Stage III, showing filaments of anterior region of gill.

Text-fig. 30. Transverse section through prodissoconch-larva at beginning of Stage III, showing gill in course of differentiation into filaments.

Text-fig. 31. Transverse section through prodissoconch-larva at beginning of Stage III, showing posterior region of gill, which has just begun to differentiate into filaments.

stage, however, this ganglion, having completed the incorporation between the pleural and cerebral ganglia, assumes a somewhat elliptical outline, with the long axis perpendicular to the stomodaeum, and to some extent has a wider ventral pleural part and a narrower dorsal cerebral part (Text-figs. 15 & 32). The larval cerebro-pleural ganglion, in this condition,—*i. e.* after the completion of the incorporation of both ganglia,—is then termed the cerebral ganglion.

*The Pedal Ganglia.* The paired pedal ganglia, originating together with the byssal invaginations on the postero-ventral side of the foot of the fetal larva, come to lie in the dorsal region of the foot of the prodissoconch-larva, just beneath the junction between the intestine and the stomach. They, also, indicate the ganglionic differentiation into the peripheral nuclear and central fibrous parts, as in the case of the cerebral ganglion, and a short commissure appears at their adjacent sides (Text-figs. 13 & 40). In the first and second stages of the larva, each pedal ganglion is somewhat spherical in shape, being slightly flattened dorso-ventrally. In the third stage, both pedal ganglia become united with each other at their adjacent faces, a bi-lobular mass of the pedal ganglia thus resulting (Text-fig. 16).

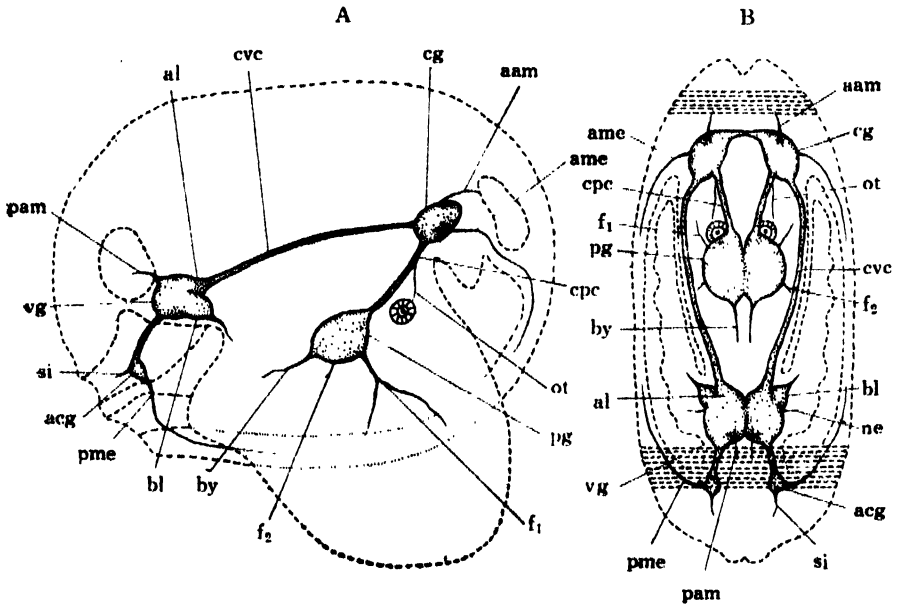
*The Visceral Ganglia.* The paired, rudimentary, visceral ganglia are first distinct in the first stage of the prodissoconch-larva, and lie in the antero-ventral region of the future posterior adductor muscle inside the rudiment of the gill (Text-fig. 43).

In the progress of development, the structure of each visceral ganglion begins to show a nuclear and a fibrous part. Each visceral ganglion shows the most complicated outline, being composed of three lobes. The antero-ventral lobe of this ganglion is called the branchial lobe, and lies at the posterior proximal base of the developing gill (Text-figs. 14 & 55). The antero-dorsal lobe is small, and is called the anterior lobe. The remaining main portion is an elliptical mass, and shows a somewhat circular outline in transverse section. These main portions on both sides of the body are united, later, with each other, at their adjacent faces (Text-figs. 17, 18 & 32).

*The Accessory Ganglia.* A small ganglionic mass, probably derived from the visceral ganglion, is found, at the end of the third stage of the larva, near either lateral wall of the cloacal chamber, and is called the accessory ganglion (Text-figs. 32 & 35), the origin of which could not be exactly traced in this investigation.

*The Nerve Connectives and the Main Nerve Fibres.* At the end of the

third stage of the larva, two pairs of nerve connectives are easily distinguishable. One pair is the cerebro-pedal connectives, each of which arises at the ventral side of the corresponding cerebral ganglion and joins the corresponding pedal ganglion at its anterior end (Text-fig. 32). The other is the cerebro-visceral connectives, each of which arises at the posterior end of the corresponding cerebral ganglion and joins the corresponding visceral ganglion at its anterior lobe (Text-fig. 32).



Text-fig. 32. Schematic representation of nervous system of prodissoconch-larva at end of Stage III, about 1 mm. long.  $\times 65$ . (A) right side view, (B) dorsal view. *aam* fibre innervating anterior adductor muscle, *acg* accessory ganglion, *al* anterior lobe of visceral ganglion, *ame* fibre innervating anterior half of mantle edge, *bl* branchial lobe of visceral ganglion, *by* fibre innervating byssal gland, *cg* cerebral ganglion, *cpc* cerebro-pedal connective, *cvc* cerebro-visceral connective, *f<sub>1</sub>* and *f<sub>2</sub>* fibres innervating foot region, *ne* fibre innervating external orifice of kidney, *ot* fibre innervating otocyst, *pam* fibre innervating posterior adductor muscle, *pg* pedal ganglion, *pme* fibre innervating posterior half of mantle edge, *si* fibre innervating siphon, *vg* visceral ganglion.

Besides these connectives, the differentiation of the nerve fibres by degrees becomes clearer. Every ganglion gives off a number of nerve fibres which innervate several organs (Text-fig. 32). From each cerebral

ganglion issue nerve fibres, innervating the anterior adductor muscle, the anterior part of the visceral sac, and the anterior half of the mantle edge on either side of the body. Each pedal ganglion gives off several nerve fibres, innervating the foot. The nerves, which arise at each visceral ganglion, are the branchial nerve and a number of nerves, innervating the posterior half of the visceral sac, the posterior adductor muscle, the cloacal chamber, the siphons, and the posterior half of the mantle edge. The small branches of nerve fibres in each organ cannot be described in detail in the present paper.

## 2. SPECIAL SENSE ORGANS, LABIAL PALPS, AND BYSSAL AND MUCOUS GLANDS

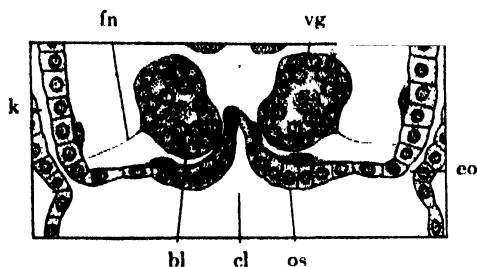
*The Otocysts.* The formation of each otocyst is observed in the first stage of the prodissoconch-larva. The body wall on either side of the body, lying postero-ventrally to the pleural ganglion, is invaginated into the foot. These paired small invaginations of the body wall are the rudiments of the otocysts. Each rudimentary otocyst deepens and, finally, forms a vesicle, its outer opening being closed. This vesicle sinks further into the interior of the foot towards the pedal ganglion (Pl. XXXVI, Fig. 8 a).

When the larva reaches the second stage, the otolith appears in each otocyst (Pl. XXXVII, Fig. 11 c, Pl. XXXVIII, Fig. 12 b, & Text-fig. 23). The otocyst at the end of the third stage is suspended in the connective tissue just anterior to the pedal ganglion, forming the vesicle of a unicellular layer. This vesicle is invested by a thin capsule of connective tissue cells (Text-fig. 24). In a live specimen in this stage, the otolith is a small, spherical concretion in the cavity of the otocyst, and rotates supported by numerous cilia.

*The Osphradia.* In the third stage of the larva, closely paired, epithelial thickenings are found at the roof of the cloacal chamber (Text-figs. 17 & 18). Each thickening of this sort consists of cubical cells, and lies in contact with the branchial lobe of the visceral ganglion, being lined with a distinct basement membrane of the thickening, though the nerves there are not yet found. This developing sense organ is the rudiment of the osphradium (Text-fig. 33).

*The Labial Palps.* In the second stage of the larva, the body wall on either side of the mouth just ventral to the pleural ganglion, thickens and is thrust out to the mantle cavity. This is the rudiment of the

labial palp (Text-fig. 25). In the third stage, each rudimentary labial palp is divided into the outer and inner palps, owing to its own growth and to the occurrence in it of a longitudinal folding. The inner surface of the outer palp and the outer surface of the inner palp consist of cubical cells, and are furnished with fine cilia (Text-fig. 26). Each outer palp becomes, finally, continuous to the body wall anterior to, and each inner palp to that posterior to, the mouth. The outer surface of the outer palp and the inner surface of the inner palp consist of non-ciliated, flattened cells. The ciliated groove between the outer and inner palps on either side of the body is continuous to the mouth.



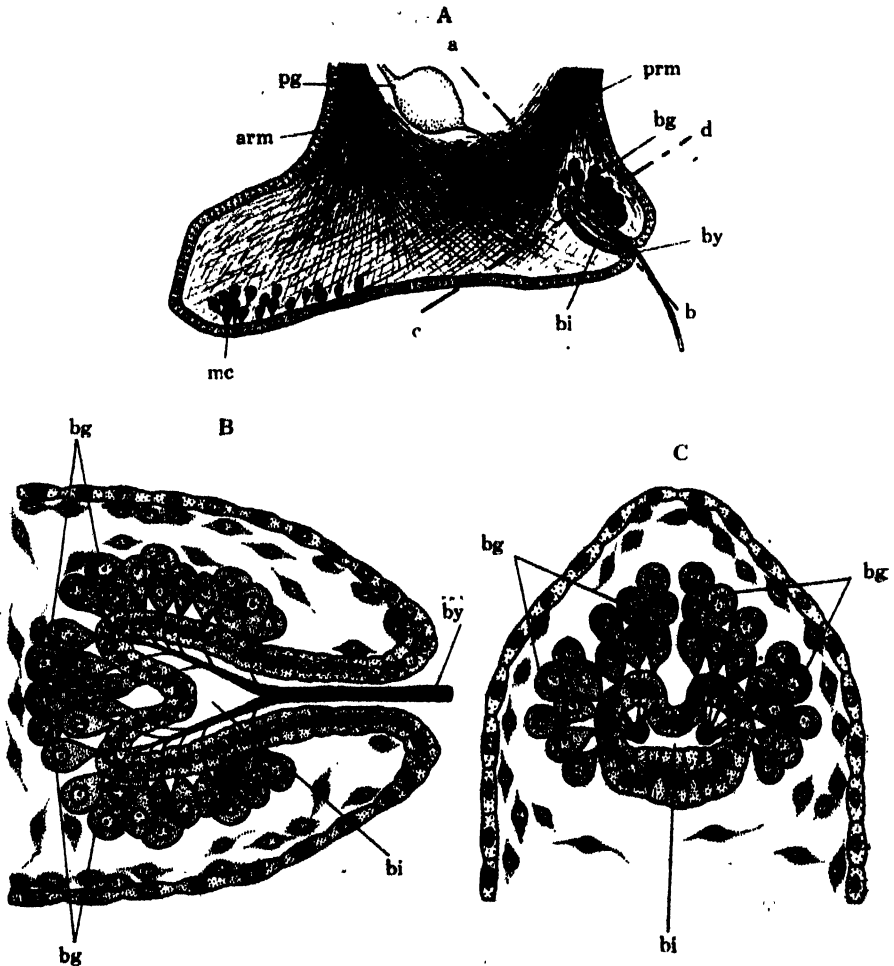
Text-fig. 33. Transverse section of prodissococonch-larva, about 1.5 mm. long, showing relation between osphradia and visceral ganglia.  $\times 200$ . *bl* branchial lobe of visceral ganglion, *cl* cloacal chamber, *eo* external orifice of kidney, *fn* nerve fibre innervating external orifice of kidney, *k* kidney, *os* osphradium, *vg* visceral ganglion.

*The Byssal Gland.* The byssal invaginations on both sides of the postero-ventral region of the foot were united to each other at their proximal end in the final stage of the fetal larva. At the first stage of the prodissococonch-larva, this united invagination shows a V-shaped lumen, the binate distal ends of it being sunk antero-dorsally into the interior of the foot (Pl. XXXVI, Figs. 8 c & 10). The wall of this lumen begins to differentiate into that of the byssal gland, though the byssus is not yet to be found. When the larva reaches the end of the second stage, the united portion of the byssal invagination increases in depth, and the lumen assumes thus a Y-shape. The byssal glandular cells begin to differentiate from the lining epithelium of the byssal invagination, and occupy a position outside this invagination on the dorso-lateral sides near its distal end. The secreted byssus begins to appear (Text-fig. 19) and the practically completed byssal glandular cells can be seen in the third stage of the larva (Text-figs. 20-22). The secretion from these glandular cells is accumulated into both tops of the Y-shaped lumen and forms a byssus-thread (Text-fig. 34).

*The Mucous Glands.* The mucous is secreted by several mucous glands as in the other lamellibranchia. The mucous-secretory cells are first distinct at the end of the third stage of the prodissococonch-larva. They



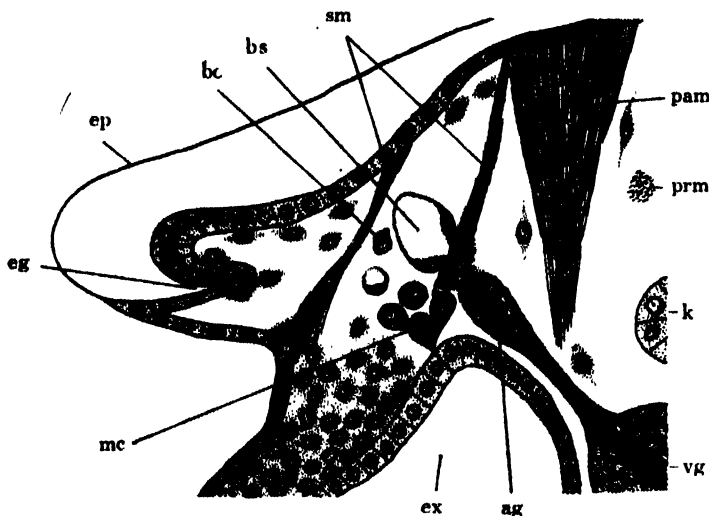
are large ovoid cells, containing, respectively, a vesicular nucleus and a number of fine granules. Their cytoplasm takes the aniline blue in Mallory's connective tissue stain. Fine granules contained in them are accurately demonstrated in the preparation stained with Heidenhain's haematoxylin. The mucous-secretory glandular cells are found grouped in three different positions, *viz.* at each lateral wall of the cloacal chamber,



Text-fig. 34. Sections, showing full-developed byssal gland. (A) Schema viewed from right side,  $\times 100$ , (B) section through *ab* in (A),  $\times 400$ , (C) section through *cd* in (A),  $\times 400$ . *arm* anterior retractor muscle of foot, *bg* byssus-secretory glandular cells, *bi* Y-shaped lumen of byssal invagination, *by* secreted byssus, *mc* mucous-secretory cells, *pg* pedal ganglion, *prm* posterior retractor muscle of foot.

at each mantle edge, and at the foot.

A group of mucous-secretory cells is found, just ventral to the accessory ganglia (Text-fig. 35). Beside this, a number of glandular cells of



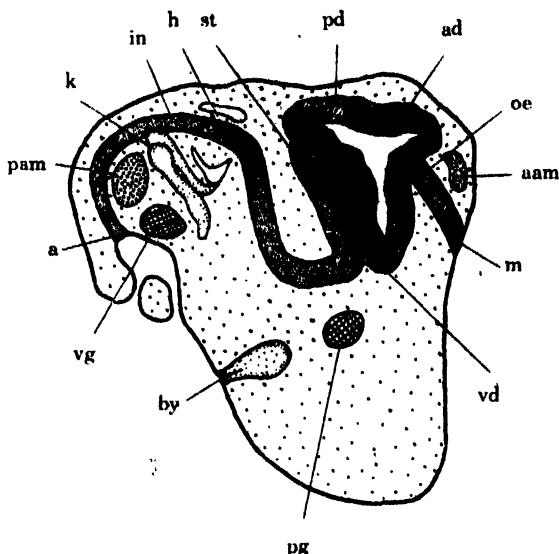
Text-fig. 35. Horizontal section through anus of prodissoconch-larva at end of Stage III, illustrating mucous gland of cloacal chamber.  $\times 300$ . *ag* accessory ganglion, *bc* blood cells, *bs* blood vessel, *eg* epidermal gland, *ep* epidermis of shell, *ex* exhalant siphon, *k* kidney, *mc* mucous-secretory cells, *pam* posterior adductor muscle, *prm* posterior retractor muscle of foot, *sm* muscles of siphon, *vg* visceral ganglion.

this sort are, also, scattered along the inner side of the mantle edge (Text-fig. 5). The other group of the mucous cells is observed at the foot. These mucous cells are found in the interstices between the muscle fibres, attached to the inner surface of the external epithelium of the foot. They are most abundant in the antero-ventral and both lateral surfaces of the foot, but decrease in number posteriorly and dorsally (Text-figs. 27 & 34).

### 3. DIGESTIVE SYSTEM

*The Alimentary Canal.* In the first stage of the prodissoconch-larva, the anterior end of the rudimentary stomach extends anteriorly and forms the so-called oesophagus, which, in turn, combines with the short ectodermal stomodaeum. The rudimentary stomach begins to expand in the antero-ventral direction, and its posterior end comes to lie at the dorsal

region of the pedal ganglia, and continues to the intestine, which makes a sharp postero-dorsal bend at the junction with the rudimentary stomach. The intestine runs further posteriorly, passes through the heart rudiment,



Text-fig. 36. Schema illustrating digestive system in median, sagittal section of prodissoconch-larva in Stage II, about 0.4 mm. long.  $\times 150$ . *a* anus, *aam* anterior adductor muscle, *ad* anterior lobe of digestive diverticulum, *by* byssal gland, *h* heart, *in* intestine, *k* kidney, *m* mouth, *oe* oesophagus, *pam* posterior adductor muscle, *pd* posterior lobe of digestive diverticulum, *pg* pedal ganglion, *st* stomach, *vd* ventral lobe of digestive diverticulum, *vg* visceral ganglion.

crystalline style sac is a little larger than that of the stomach proper, and its wall begins to be covered with stout cilia. A mass of secretion granules is now found in the dorsal part of this rudimentary sac (Text-fig. 37). This secretion mass is the crystalline style in its original condition, that seems to be a product of some cells constituting mainly the dorsal wall of the vertical ridges mentioned above. In most cases of the present writer's material, only a small concretion of a rudimentary crystalline style was seen. In the third stage, the secretion mass can be first called a crystalline style, and forms a long rod, which extends to the posterior portion of the stomach.

Later than the second stage of the larva, both right and left halves

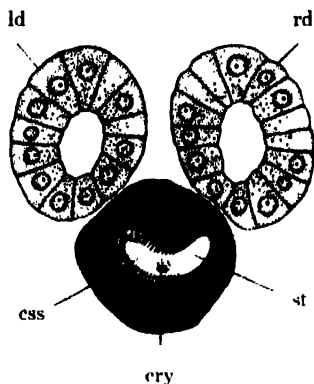
and then continues to the anus (Pl. XXXVI, Fig. 10 & Text-fig. 36).

In the second stage of the larva, the structure of the wall of the rudimentary stomach is, also, undergoing further differentiation. Along the anterior and posterior median walls of the rudimentary stomach, two slight ridges of the lining epithelium, anterior and posterior, respectively, form there vertically, and the rudimentary stomachal cavity tends to divide into the right and left halves.

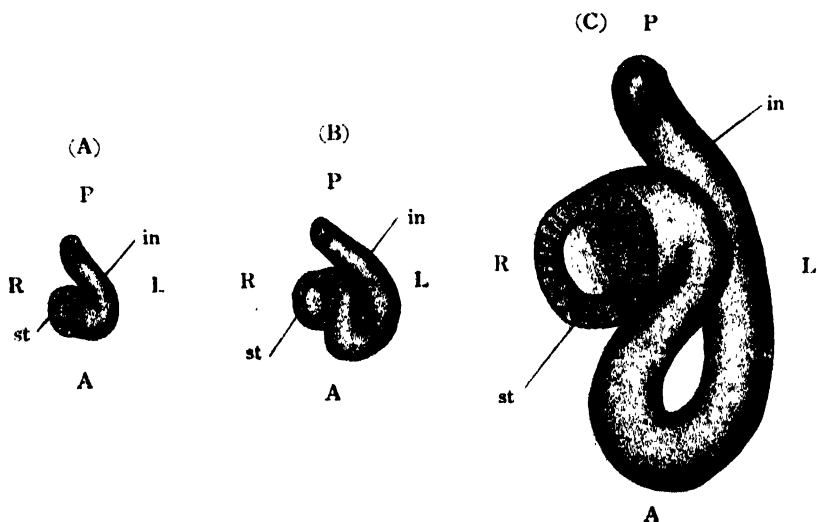
The right cavity develops into the adult stomach, and the left into the future sac of the crystalline style. The cavity of this rudimentary

of the rudimentary stomachal cavity, mentioned already, diminish gradually in dimension near their posterior end and are continuous to the tubular lumen of the intestine, there being no clear distinction between the stomach and the intestine in the structure of both walls. At the same time, a noticeable change is taking place in the anterior course of the intestine. In the first stage of the prodissoconch-larva, the bending at the junction between the intestine and the stomach was actually a sharp turn directed dorso-posteriorly (Text-fig. 38 a). In the subsequent stages, this portion of the intestine comes to form a loop at the dorsal side of the pedal ganglia (Text-fig. 38 b & c).

In the third stage, the intestine, beginning at the posterior end of the comparatively swollen stomach, proceeds first dextro-



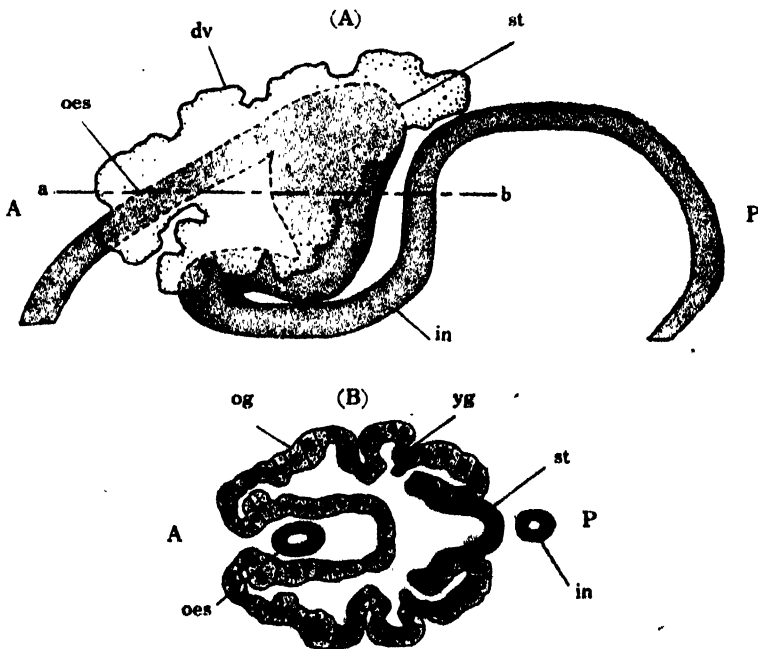
Text-fig. 37. Horizontal section through stomach and digestive diverticula, showing origination of crystalline style of prodissoconch-larva, at beginning of Stage II.  $\times 400$ . *cry* crystalline style, *css* crystalline style sac, *ld* left digestive diverticulum, *rd* right digestive diverticulum, *st* stomach proper.



Text-fig. 38. Reconstructive schema, showing anterior portion of intestine in three developmental stages of prodissoconch-larva, viewed from dorsal.  $\times 150$ . (A) from larva, about 0.3 mm. long, (B) from larva, about 0.4 mm. long, (C) from larva, about 1 mm. long. *A* anterior, *L* left, *P* posterior, *R* right; *in* intestine, *st* stomach.

anteriorly, turns sinistrally, and is directed then posteriorly. Then it continues a postero-dorsal course, underneath the stomach, shifting slightly to the right side of the body, and reaches as high as the level of the dorsal surface of the stomach. It proceeds, finally, postero-ventrally, along the median plane of the body and passes through the heart.

*The Digestive Diverticula.* Each rudimentary digestive diverticulum, evaginated on either side of the anterior end of the rudimentary stomach at the final stage of the fetal larva, is increasing in size and differentiating into a glandular structure, with the progress of the development of the prodissoconch-larva (Pl. XXXVI, Figs. 8 a & 9). In the second stage, each digestive diverticulum divides into three lobes—an anterior, a posterior and a ventral (Text-fig. 36, Pl. XXXVII, Fig. 11 c, & Pl. XXXVIII, Fig. 12 b & c). In the third stage, each lobe of the digestive diverticulum subdivides into lobules, and comes, finally, to have an alveolar external appearance (Text-fig. 39 a).



Text-fig. 39. Digestive tract and diverticula of prodissoconch-larva at end of Stage III, about 1.2 mm. long.  $\times 100$ . (A) digestive tract and diverticulum as seen from left side, (B) horizontal section through *ab* in (A). *A* anterior, *P* posterior; *dv* digestive diverticulum, *in* intestine, *oes* oesophagus, *og* older cells of digestive diverticulum, *st* stomach, *yg* younger cells of digestive diverticulum.

The wall of the digestive diverticulum consists of glandular cells which are different from those of other portions of the alimentary system. In the third stage, the glandular cells of the digestive diverticulum have a high differentiation, this indicating the several degrees of cell activity (Text-figs. 8 & 39 b). The most highly differentiated older cells are larger and longer, containing many granules. The younger cells, differentiated imperfectly, are smaller and shorter, containing dense cytoplasm deeply stainable, and lie, mainly, near the tip and base of each lobule.

#### 4. MUSCULAR SYSTEM

In the development of the prodissoconch-larva, some of the mesenchyme cells, which were scattered widely in the body of the fetal larva, are differentiating into myocytes, and form muscle fibres.

*The Muscles of the Foot.* A small number of myocytes were found in the foot of the fetal larva (OKADA 1936). In the first stage of the larva, a large number of myocytes are found attached inside the superficial ectoderm of the foot, mainly on the ventral and lateral sides. Besides these, a small number of myocytes are scattered in the interior of the foot (Text-fig. 40). In the third stage of the larva, the foot is merely a muscular protrusion, and the muscles of the foot are grouped into the right and left halves by a median cleft (Text-fig. 41). Each half of them is composed of interlaced muscle fibres running in all directions.

*The Anterior Adductor Muscle.* In the second stage of the larva, a number of spindle-shaped cells, forming a group, appear antero-dorsally to the cerebral ganglion near the anterior end of the visceral sac (Pl. XXXVII, Fig. 11 a & Text-fig. 42). These cells increase in number and differentiate into the anterior adductor. In the third stage, the anterior adductor forms a stout bundle of muscle fibres (Text-fig. 15), which run, transversely, along the anterior side of the cerebral ganglia. It is irregularly elliptical in sagittal sections of the body (Text-figs. 3, 32 & 36).

*The Posterior Adductor Muscle.* At nearly the same stage, when the rudiment of the anterior adductor is seen, there is also found that of the posterior adductor at the postero-dorsal side of the visceral ganglia near the posterior end of the visceral sac. In the third stage, the posterior adductor forms, also, a stout bundle of muscle fibres near the posterior end of the visceral sac (Text-fig. 44), and shows an irregular oval shape in sagittal sections of the body (Text-figs. 3, 32 & 36).

*The Anterior Retractor Muscles of the Foot.* In the second stage of

the larva, a little later than the appearance of the rudiments of the anterior and posterior adductors, on either side of the body, several myocytes are arranged in a slender strand, arising from the dorsal side of the anterior adductor and running postero-ventrally into the foot, through the inside of the cerebro-pleural ganglion (Text-fig. 45). In the third stage, each anterior foot retractor is a narrow, long bundle of muscle fibres. Each bundle converges once at the inside of the respective cerebro-pleural ganglion, and then diverges widely into the foot (Text-figs. 34 a & 49). It is circular in transverse sections of this muscle.

*The Posterior Retractor Muscles of the Foot.* Almost at the same stage, as the appearance of the anterior foot retractor, the strand of each posterior foot retractor is, also, indicated near the rudimentary posterior adductor (Text-fig. 43). In the third stage of the larva, the posterior foot retractor is a bundle of muscle fibres, as in the case of the anterior foot retractor (Text-fig. 46). It arises from the dorsal side of the posterior

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Text-figs. 40, 42-45, 47 and 48 are photographed from specimens stained following Mallory's connective tissue staining method and Text-figs. 41 and 46 from specimens stained with Heidenhain's haematoxylin.  $\times 300$ . *aa* anterior adductor muscle, *ar* anterior retractor muscle of foot, *bl* levator branchiarum muscle, *ef* epithelium of foot, *f* foot, *g* gill, *in* intestine, *k* kidney, *lp* labial palp, *mf* muscle fibres of foot, *pa* posterior adductor muscle, *pg* pedal ganglion, *plg* pleural part of cerebro-pleural ganglion, *pr* posterior retractor muscle of foot, *vg* visceral ganglion.

Text-fig. 40. Transverse section through prodissocoench-larva in Stage I, showing pedal ganglia and myocytes in foot.

Text-fig. 41. Transverse section through prodissocoench-larva in Stage III, showing muscles of foot.

Text-fig. 42. Transverse section through prodissocoench-larva in Stage II, showing myocytes of anterior adductor muscle and rudiments of labial palps.

Text-fig. 43. Transverse section through prodissocoench-larva in Stage II, showing myocytes of posterior adductor muscle, visceral ganglia, and rudimentary gills.

Text-fig. 44. Horizontal section through prodissocoench-larva in Stage III, showing posterior adductor muscle and posterior retractor muscles of foot.

Text-fig. 45. Transverse section through prodissocoench-larva in Stage II, showing myocytes of anterior foot retractor muscle and pleural part of cerebro-pleural ganglion.

Text-fig. 46. Horizontal section through prodissocoench-larva at end of Stage III, showing posterior foot retractor muscle and visceral ganglion.

Text-fig. 47. Transverse section through prodissocoench-larva at beginning of Stage II, showing origination of levator branchiarum muscle.

Text-fig. 48. Transverse section through prodissocoench-larva at end of Stage III, showing levator branchiarum muscle.







adductor and diverges into the foot, after passing through the posterior side of the kidney, and converging once at the inside of the respective visceral ganglion (Text-figs. 18, 34 a, 44 & 46).

*The Pallial Muscles.* In the third stage of the larva, a number of slender muscles, which are, as a whole, called the pallial muscles, are found regularly distributed in the whole length of the marginal portion of the mantles. These muscles are derived from the mesenchymal elements situated near the mantle edge in the first and second stages, and are specified into two groups. One is that of the longitudinal muscle fibres running parallel to the mantle edge (Text-fig. 5 b). The other is that of the transverse muscles, which arise from the upper, outer side of the pallial portion and run obliquely towards the mantle edge, and which form the outer and inner sets (Text-fig. 5 b). The outer set reaches the depression of the epidermal gland, and the inner set the longitudinal

Text-figs. 49 and 52-57 are photographed from specimens stained following Mallory's connective tissue staining method, and Text-figs. 50 and 51 from specimens stained with Heidenhain's haematoxylin.  $\times 300$ . *a* auricle of heart, *ar* anterior retractor muscle of foot, *br* branchial lobe of visceral ganglion, *bv* blood vessel, *cg* cerebral part of cerebro-pleural ganglion, *cl* cloacal chamber, *dv* digestive diverticulum, *ep* epidermal gland, *es* exhalant siphon, *g* gill, *gc* germ cells, *go* gonad, *h* heart, *in* intestine, *k* kidney, *lp* labial palp, *ok* outer orifice of kidney, *os* osphradium, *p* pericardial cavity, *pk* urino-pericardial opening, *pm* pallial muscle, *sm* muscle of siphon, *v* ventricle of heart, *vg* visceral ganglion.

Text-fig. 49. Transverse section through prodissoconch-larva at end of Stage III, showing anterior retractor muscle of foot, labial palp and cerebro-pleural ganglion.

Text-fig. 50. Horizontal section through prodissoconch-larva at end of Stage III, showing epidermal gland, pallial muscle and blood vessel.

Text-fig. 51. Transverse section through prodissoconch-larva in Stage III, showing osphradia and muscle of siphon.

Text-fig. 52. Horizontal section through prodissoconch-larva in Stage II, showing heart and pericardium.

Text-fig. 53. Sagittal section through prodissoconch-larva in Stage II, showing heart, pericardium, and urino-pericardial opening of kidney.

Text-fig. 54. Transverse section through prodissoconch-larva in Stage III, showing ventricle and auricles of heart.

Text-fig. 55. Transverse section through prodissoconch-larva in Stage II, showing external orifice of kidney and branchial lobe of visceral ganglion.

Text-fig. 56. Transverse section through prodissoconch-larva in Stage II, showing primordial germ cells.

Text-fig. 57. Horizontal section through prodissoconch-larva in Stage III, showing cell-multiplication in gonad.

muscles at the mantle edge (Text-fig. 50).

*The Muscles of the Siphons.* As already stated, the siphons are formed by a union of both mantle edges at the end of the third stage of the larva. Consequently, the muscular wall of the siphonal tubes is merely a modification of longitudinal pallial muscles. Besides this muscular tissue, in the siphonal tubes, another set of muscle strands are marked at both lateral walls of the cloaca, arising at the outer side of the mantle beneath the posterior adductor and passing obliquely into the base of the siphons (Text-fig. 51). These muscle strands on either side of the siphons are, also, merely modifications of the transverse pallial muscles at the posterior end of the mantle.

*The Levator Branchiarum Muscles.* After the larva reaches the second stage, a set of slender muscle strands is found at the anterior side of the pericardium, suspending the gill to the dorsal body wall near the ligament of the shell (Text-figs. 47 & 48). These muscle strands are so slender and short, that many investigators have failed to notice them, and they may be called the 'levator branchiarum muscles.'

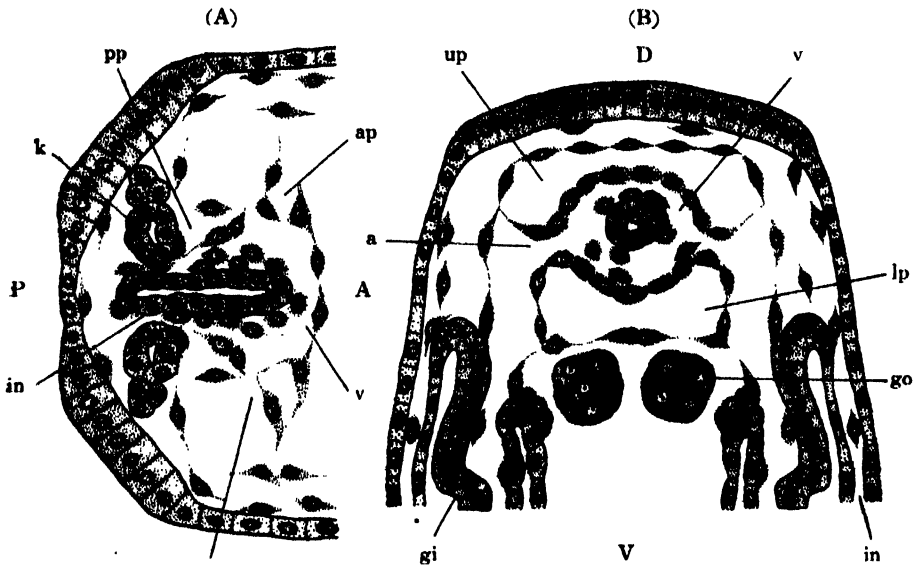
## 5. CIRCULATORY SYSTEM

*The Heart and the Pericardium.* In the final stage of the fetal larva, the origin of the heart and pericardium was indicated by some cells, which were members of the teloblastic cell-masses on both sides of the body near its posterior end. In the first stage of the prodissoconch-larva, these cells acquire a spindle shape and are rearranged, for the first time, surrounding a distinct cavity on either side of the intestine (Pl. XXXVI, Figs. 8 c, 9 & 10). The outer wall of this cavity forms the future pericardium and the inner wall the future ventricle of the heart, the cavity itself being the rudimentary pericardial cavity.

The process of the formation of the heart and pericardium finishes, seemingly, in a very short time. In reality, in the second stage, when the larva attains 0.4 mm. in length, the rudimentary heart, with rudimentary auricles and pericardium, is already visible (Text-figs. 28, 52 & 58). The process of the formation of the heart and pericardial cavity may, thus, be explained as follows: At first, on either side of the intestine, the outer (pericardial) and the inner (ventricular) walls approach each other, and form a bridge, perpendicular to the axis of the heart rudiment, at the level of the middle 'circumference' of the rudimentary pericardial cavity. By this bridge, the upper and lower divisions of the cavity are established.

These divisions communicate with each other in the spaces anterior and posterior to the bridge. In the immediately succeeding stage, on both sides of the intestine, the ventral ends of the lower divisions of the rudimentary pericardial cavities become first united with each other on the ventral side of the intestine, and then the dorsal ends of the upper divisions become united on the dorsal side of the intestine.

At the end of the second stage of the larva, the completion of the pericardial cavity, mentioned above, results in the formation of the rudi-



Text-fig. 58. Sections through early prodissoconch-larva, showing structure of heart and pericardium.  $\times 350$ . (A) horizontal section of larva in Stage I, about 0.3 mm. long, (B) transverse section of larva in Stage II, about 0.45 mm. long. *a* auricle of heart, *ap* anterior half of pericardial cavity, *gi* gill, *go* gonad, *in* intestine, *k* kidney, *lp* lower half of pericardial cavity, *m* mantle, *pp* posterior half of pericardial cavity, *up* upper half of pericardial cavity, *v* ventricle of heart.

mentary heart. The inner wall of this cavity surrounds the intestine, with one break at the portion of the bridge on either side of it. A number of mesenchymal cells are found in a narrow space between this inner wall and the intestine. This narrow space grows in the course of time, and the inner wall of the pericardial cavity forms a chamber round the intestine. Both lateral bridges are differentiated into the lateral passages of this chamber, the practically completed heart being thus formed (Text-fig. 58).

The pericardium at this point becomes a thin cell-layer surrounding the large, practically completed, pericardial cavity of a nearly inverted conical shape, in which the heart is suspended. Each of the kidneys is open on either side of the narrower postero-ventral end of the conical cavity (Text-fig. 53).

In the third stage, the walls of the heart chamber surrounding the intestine thicken and become somewhat muscular, while the walls of both lateral passages are composed of thin, non-muscular elements. The ventricle of the adult heart is shown by this chamber, and the auricles by both lateral passages (Text-fig. 54).

*The Blood Vessels.* In the third stage of the larva, the main blood vessels and sinuses are observed. Two large vascular trunks, an anterior and a posterior artery, arise, respectively, at the anterior and the posterior end of the ventricle. The anterior artery, starting at the dorsal side of the intestine, runs anteriorly along the median dorsal line of the visceral sac, and branches into several small arteries, lying distributed in the anterior half of the visceral sac, in the anterior half of the mantle, and in the foot region. The posterior artery, starting on the ventral side of the intestine, runs posteriorly towards the posterior side of the kidneys, and divides into the right and left branches, each branch extending to the posterior half of the respective mantle. The walls of these blood vessels consist of a single layer of flattened cells (Text-figs. 35 & 50), being embedded in scattered mesodermal elements. Each auricle extends ventro-laterally below the respective kidney, and is continuous with the blood sinus of the respective gill.

In the present investigation, the vascular system has not been further minutely traced. In a live specimen in the third stage, the pulsation of the heart and circulation of the blood are dimly observed through the prodisoconch.

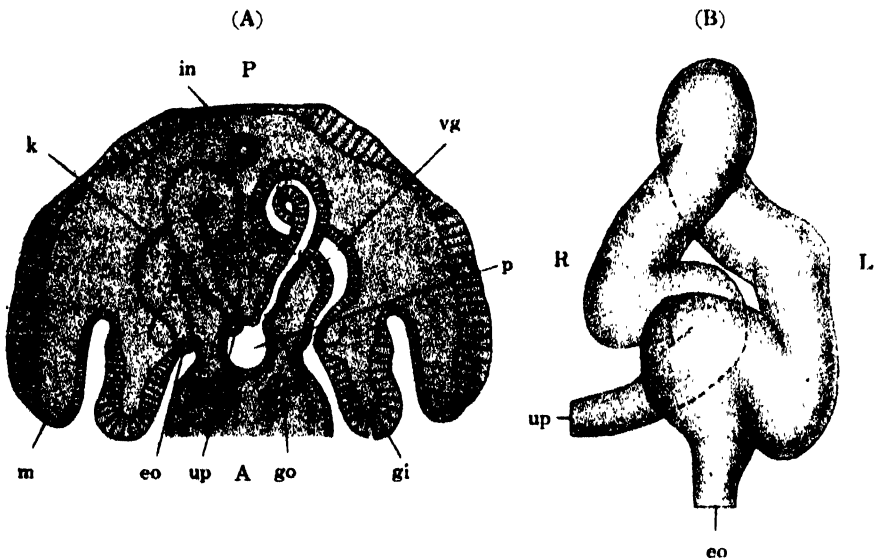
*The Blood Corpuscles.* The blood corpuscles already are found at the end of the third stage of the prodisoconch-larva. They are probably differentiated from the mesenchymal cells, but the present writer cannot speak positively regarding their origin.

## 6. EXCRETORY SYSTEM

*The Protonephridia.* In the final stage of the fetal larva, the protonephridium was found on either side of the anterior portion of the body (OKADA 1936). This is a vestigial organ differentiated only in the fetal

larva and it becomes degenerated in the beginning stage of the prodissococonch-larva. This kind of organ is never found in the later stages. Thus, the protonephridium is merely a larval excretory organ which is functional before the completion of the true excretory organ (Pl. XXXVI, Fig. 8 a).

*The Excretory Organs.* Each of the paired definite excretory organs is called the kidney. This organ is originally composed of a number of cells of the teloblastic cell-mass at the respective side of the body. At the beginning of the first stage of the prodissococonch-larva, the rudimentary kidney is first indicated as a cell-mass, lying at the postero-ventral side of the respective rudimentary pericardial cavity. This cell-mass acquires a



Text-fig. 59. Reconstructive schema of kidney in early prodissococonch-larva.  $\times 300$ . (A) horizontal section of larva, about 0.35 mm. long, (B) left kidney of larva, about 0.6 mm. long, as seen from anterior. A anterior, D dorsal, L left, P posterior, R right, V ventral; eo external orifice of kidney, gi gill, go gonad, in intestine, k kidney, m mantle, p pericardial cavity, up urino-pericardial opening, vg visceral ganglion.

tubular arrangement in conformity with the formation of the rudimentary heart and pericardium. The proximal end of this tubule is continuous to the respective postero-ventral side of the pericardial cavity. This junction represents the urino-pericardial opening. Therefrom, the nephridial tubule advances in a postero-dorsal direction and reaches the respective lateral side of the posterior portion of the intestine. At this point, the tubular organ is bent down and undergoes convolutions (Text-fig. 59), and then

runs to the anterior side of the respective visceral ganglion, just inside it (Text-fig. 55).

Thus, each of the early kidneys is a convoluted, tubular duct lined by a single layer of cubical cells. In fixed specimens, a number of long cilia are found only at the proximal end of the kidney, or the urino-pericardial duct, but no cilia are observed covering all the other parts. The lumen of the kidney is somewhat narrow near the urino-pericardial duct and near the external orifice. It is most spacious in the convoluted portion, especially in the final convolution to the external opening (Text-fig. 59). The convolutions of the kidney become more advanced and complete in the third stage of the prodissoconch-larva.

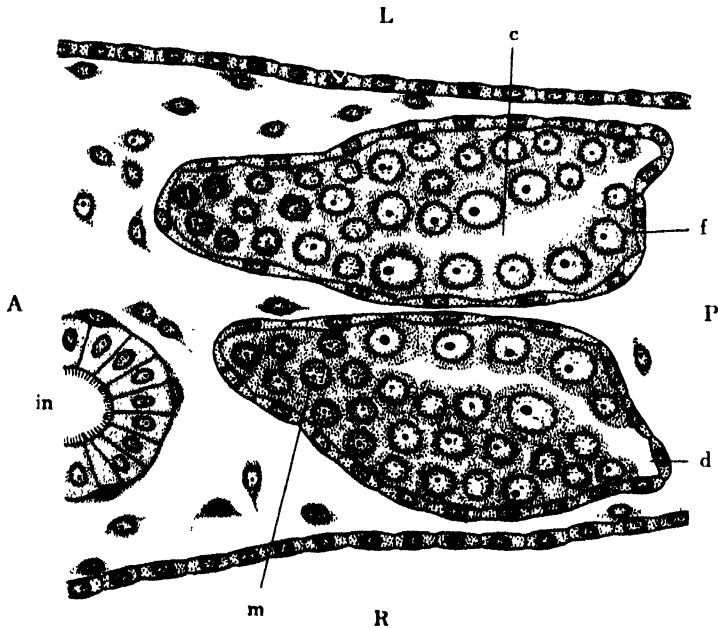
## 7. REPRODUCTIVE SYSTEM

Primordial germ cells on either side of the later fetal larva, lying on the antero-ventral side of the respective teloblastic cell-mass, pass through a period of inactivity for a short time, until the formation of the gonad begins to occur. Because, no change in the number and appearance of the primordial germ cells themselves are observed in the first stage of the prodissoconch-larva (Pl. XXXVI, Figs. 8 c & 9, & Pl. XXXVII, Fig. 11 e).

When the larva reaches the second stage, the primordial germ cells, forming a mass on either side of the body, come to lie at the region antero-ventral to the pericardium, and begin to increase in number. This mass of primordial germ cells meets one of the opposite side at its median surface. Such a definite position of the germ cells is determined, by the developmental changes of the visceral sac, which occur with the formation of the gill and with the differentiation of the teloblastic cell-masses (Pl. XXXVII, Fig. 12 d, & Text-figs. 28, 56 & 58). After the formation of the rudimentary kidneys, heart, and pericardium are finished, the germ cells begin to separate from the pericardial lining, with which they have hitherto grown together, and form a pair of masses of the gonad. Each gonad is a solid mass surrounded by the so-called peritoneal cells, which are the modified mesenchymal elements, and presents a kidney-shape. Following the gonad formation, the germ cells increase in number, and there mitotic figures are often seen (Text-fig. 57). Thus, the gonad itself increases in size and becomes elongated antero-dorsally.

At the end of the third stage of the larva, the next developments occurring in relation to the gonad are the separation of the male and

female regions and the formation of the external duct. Each initial gonad, elongated antero-dorsally, lies near the posterior lobe of the respective digestive diverticulum and with the posterior wider portion has a club-shaped outline. The narrower antero-lateral portion continues to extend and grows to form the lobe of the male region. This separation of the male region is predominant in the left gonad, perhaps owing to less resistance to the growth, which is affected by the dislocation of the intestine to the right side of the body (Text-fig. 60). In the wider postero-ventral



Text-fig. 60. Horizontal section of prodissococonch-larva at end of Stage III, about 1.5 mm. long.  $\times 350$ . *A* anterior, *L* left, *P* posterior, *R* right; *c* central cavity of female region of gonad, *d* external duct of gonad, *f* female region of gonad, *in* intestine, *m* male region of gonad.

portion, the germ cells are rearranged in a single layer surrounding a cavity in the centre. These elements grow so as to develop the oögonia. The male cells constituting the antero-lateral portion of the gonad are smaller, each containing a spherical nucleus. Chromatin strands in a nucleus of the male cell compose a reticular structure furnished with small nucleoli. On the other hand, the female cells constituting the posterior portion of the gonad are larger, each having a large spherical



nucleus, which contains a distinct nucleolus and a few peripheral chromatins.

The peritoneal lining is thicker at the outer and thinner at the inner side of each gonad. At the postero-ventral end of the gonad, this peritoneal lining extends in a postero-ventral direction, and forms a narrow tubule. This tubule is continuous to the central cavity of the gonad. The first indication of the external duct of the gonad is formed by this process. The establishment of the external orifice seems to occur after the larvae are extruded from the mother.

#### REMARKS ON POST-LARVAL DEVELOPMENT, IN COMPARISON WITH LAMELLIBRANCHIA IN GENERAL

The larval form in most marine Lamellibranchia is usually the well known 'veliger.' In the progress of development, this veliger is metamorphosed into the initial form of bivalve called 'post-larva,' owing to the degeneration of the velum and the related larval organs. The changes in the form and structure in the veliger and post-larval stages of marine bivalves have been investigated in detail in the case of the following species: *Ostrea virginiana* (BROOKS 1880), *Ostrea edulis* (HORST 1882), *Entovalva mirabilis* (VOELTZKOW 1892), *Dreissensia polymorpha* (MEISENHEIMER 1901 a), *Pecten tenuicostatus* (DREW 1906), *Xylotrya gouldi*, *Teredo navalis*, and *Teredo dilatata* (SIGERFOOS 1908), and several other species (LOVÉN 1884, MIYAZAKI 1935 & 1936).

The typical 'veliger' does not, however, appear in the larval stage of a few marine Lamellibranchia. In the Protobranchia, the development of *Nucula proxima* and *Yoldia limatula* passes through a stage of modified trochophore, which the term veliger may not be applied because of the incomplete velum (DREW 1899), and the development of *Nucula delphinodonta* is almost direct, because its larval form is a degenerated trochophore with feeble cilia on the whole surface of the body (DREW 1901). A few species of the marine Eulamellibranchia, also seem to pass through no typical veliger stage in their development.

The development of the freshwater bivalves is marked in their larval stage by a modification or a degeneration of the veliger. According to MIYAZAKI (1936), the larval form of *Corbicula* seems to be a degenerated veliger with a feeble velum, and its metamorphosis into the post-larva is less distinct than that in the case of the marine type. In the Unionidae, the first half of the development occurs in the gill of the mother, and the latter half is carried on apart from the mother. The larval form of

this mussel represents a specially degenerated trochophore without any signs of veliger, and soon undergoes a metamorphosis into a glochidium with a larval shell. After extrusion from the mother, the glochidia are propagated by fish, as external parasites, and are then transferred gradually to an adult form of bivalve. Such a development of the Unionidae has been definitely reported as occurring in several species: for example, *Unio* sp. (LILLIE 1895), *Anodonta piscinalis*, *Margaritana margaritifera*, *Unio pictorum*, and *Unio tumidus* (HARMS 1908), and *Anodonta cellensis* (HERBERS 1914).

In the Sphaeriidae, the development is highly modified from that of the marine type. ZIEGLER (1885) has described the outline of such a development in *Cyclas cornea*. In the previous (OKADA 1935 c & 1936) as well as in this paper, the present writer has noted four developmental stages of *Sphaerium japonicum*, viz. blastula, gastrula, fetal larva, and prodissoconch-larva. As already stated, the fetal larva of the species under investigation is a highly degenerated veliger without a velum. The prodissoconch-larva under discussion corresponds to the post-larva of the general marine Lamellibranchia. Moreover, in this case, the so-called metamorphosis from veliger into post-larva cannot be found, and the developmental changes of the external outline are continuous, occurring gradually. Thus, the direct development from the fetal larva to the prodissoconch-larva, which goes on without distinct metamorphosis in the present writer's species, is conspicuous and characteristic, in comparison with the development of the other Lamellibranchia. The degeneration in the veliger form seems to be caused by the non-occurrence of the free-swimming larval stage. The non-occurrence of this stage is connected with the viviparous development. Therefore, the fact that the larval types of the freshwater bivalves are modified from those of the marine bivalves, appears to be correlated with the difference in the character of their development.

The aspects of the development of the prodissoconch-larva of the species under investigation are, also, fundamentally in agreement with those of the Lamellibranchia in general, although the above-mentioned speciality, caused by the different type of development, definitely modifies them to some extent.

In the present writer's previous paper (OKADA 1936), he reported the delayed formation of the shell gland in his species in comparison with the formation in the case of Lamellibranchia in general, such as *Unio* reported by LILLIE (1895) and *Dreissensia* by MEISENHEIMER (1901 a), etc. Accord-

ingly, in the earliest stage of the prodissoconch-larva, the conch is very small and the foot very large in size relatively to the whole body. In the later stage, the whole body including the foot becomes first enveloped by the mantle and shell. While, in the case of the so-called post-larva in the Lamellibranchia in general, the larval body is already included within the mantle and shell from its earliest stage.

The gill of the Lamellibranchia is one of the most important organs from the phylogenetic point of view. Its development, therefore, has been studied by many investigators in the case of various species (MITSUKURI 1881, RICE 1897 & 1908, NOMURA 1922, etc.). In the Sphaeriidae, the development of the gill attracts attention not only from the phylogenetic stand-point, but also from the peculiar character of the breeding habits. WASSERLOS (1911) has reported, on *Cyclas cornea*, the detailed observations of development concerning the gill and related organs. In the present investigation the writer has not specially observed the development of the gill in the prodissoconch-larva of *Sphaerium japonicum*.

The organogenesis of the nervous system, digestive system, definite muscles, circulatory system, excretory system and of reproductive system of the present writer's species, is also generally in good agreement with that in many preceding reports on various species, viz. *Cyclas* by ZIEGLER (1885), *Dreissensia* by MEISENHEIMER (1901 a), *Pecten* by DREW (1906), *Teredo* by SIGERFOOS (1908) and *Anodonta* by HERBERS (1914), etc. The inner structure at the end of the third stage of the prodissoconch-larva of the species under investigation indicates nearly the same organization, as that of the adult specimen of *Sphaerium notatum* reported by MONK (1928).

In conclusion, the present investigation will elucidate the later development of the Sphaeriidae and contribute to that of the Lamellibranchia in general.

#### SUMMARY

1) The results of the observation of the later development of *Sphaerium japonicum biwaense* MORI are reported in this paper.

2) In this species, the larva, during the period, after the fetal larval stage till the delivery, is still reared in the gill of the mother, but is movable and has a prodissoconch, and consequently, is named the prodissoconch-larva.

3) In the external shape the transition is gradual, and, therefore, its

development is divided into three stages, mainly according to size, *i. e.* the first stage 0.25–0.3 mm., the second stage 0.3–0.5 mm. and the third stage more than 0.5 mm. in antero-posterior length.

4) In the first stage of this larva, the mantles, the gills, the otocysts, the heart, the pericardium, and the kidneys begin to form.

5) In the second stage, the mantles and the shell gland extend, and the shell-ligament, the labial palps, the crystalline style, the gonads and the definite muscles begin to form.

6) In the third stage, the siphons, the mucous glands and the osphradia begin to form. The byssus appears at the beginning, and disappears at the end of this stage.

7) The gonads, the osphradia and the gills are not yet sufficiently differentiated in the final stage of the prodissoconch-larva.

8) The byssal gland is fully developed in the final stage of the larva and degenerates slowly after delivery from the mother.

9) *Sphaerium japonicum biwaense* develops directly without any metamorphosis, differing in this respect from the development of the marine Lamellibranchia in general and of the Unionidae.

10) The delivery of the prodissoconch-larva from the mother occurs at later stages, the larva measuring 1.5–2.5 mm. in antero-posterior length.

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EXPLANATION OF PLATE XXXV

- Fig. 1. Right side view of prodissoconch-larva in Stage I, about 0.25 mm. in length. Photo. from specimen stained with boraxcarmine.  $\times 150$ .  
 Fig. 2. Right side view of larva in Stage II, about 0.35 mm. in length. Photo. from specimen stained with boraxcarmine.  $\times 150$ .  
 Fig. 3. Right side view of larva in Stage III, about 0.5 mm. in length. Photo. from narcotized specimen.  $\times 20$ .  
 Fig. 4. Right side view of larva in Stage III, about 0.7 mm. in length. Photo. from fixed specimen.  $\times 20$ .  
 Fig. 5. Right side view of larva in Stage III, about 1 mm. in length. Photo. from narcotized specimen.  $\times 20$ .  
 Fig. 6. Right side view of larva in Stage III, about 1.2 mm. in length. Photo. from fixed specimen.  $\times 20$ .  
 Fig. 7. Left side view of larva in Stage III, about 0.9 mm. in length. Photo. from specimen stained with boraxcarmine.  $\times 150$ .

EXPLANATION OF PLATE XXXVI

- Fig. 8. Three transverse sections of prodissoconch-larva at Stage I.  $\times 300$ .  
 (a) through digestive diverticula and otocysts,  
 (b) through stomach and pedal ganglia,  
 (c) through primordial germ cells and byssal gland.  
 Fig. 9. Horizontal section of larva at Stage I, through cerebro-pleural ganglia and primordial germ cells.  $\times 300$ .  
 Fig. 10. Median sagittal section of larva at Stage I.  $\times 300$ .

EXPLANATION OF PLATE XXXVII

- Fig. 11. Six transverse sections of prodissoconch-larva at Stage II, about 0.3 mm. in length.  $\times 300$ .  
 (a) through foremost portion of body,  
 (b) through cerebral commissure,  
 (c) through digestive diverticula, otocysts, and pedal ganglia,  
 (d) through stomach and byssal gland,  
 (e) through gonads and orifice of byssal invagination,  
 (f) through posterior portion of body.

EXPLANATION OF PLATE XXXVIII

- Fig. 12. Five transverse sections prodissoconch-larva at Stage II, about 0.4 mm. in length.  $\times 300$ .  
 (a) through cerebro-pleural ganglia and rudimentary labial palps,

- (b) through digestive diverticula, otocysts, and pedal ganglia,
- (c) through stomach and pedal ganglia,
- (d) through gonads and orifice of byssal invagination,
- (e) through visceral ganglia and hindermost portion of gills.

#### ABBREVIATIONS IN PLATES XXXV-XXXVIII

<i>aa</i> anterior adductor muscle	<i>k</i> kidney
<i>bi</i> byssal invagination	<i>l</i> ligament of shell
<i>bl</i> levator branchiarum muscle	<i>lhp</i> lower half of pericardial cavity
<i>by</i> byssus	<i>lp</i> labial palp
<i>cc</i> cerebral commissure	<i>m</i> mantle
<i>cg</i> cerebral part of cerebro-pleural ganglion	<i>oe</i> oesophagus
<i>cpg</i> cerebro-pleural ganglion	<i>ot</i> otocyst
<i>dv</i> digestive diverticulum	<i>p</i> pericardial cavity
<i>ep</i> epidermis of shell	<i>pg</i> pedal ganglion
<i>epg</i> epidermal gland	<i>plg</i> pleural part of cerebro-pleural ganglion
<i>f</i> foot	<i>pn</i> protonephridium
<i>g</i> gill	<i>st</i> stomach
<i>gc</i> germ cells	<i>uhp</i> upper half of pericardial cavity
<i>h</i> heart	<i>vg</i> visceral ganglion
<i>in</i> intestine	

Fig. 1



Fig. 2



Fig. 5



Fig. 6



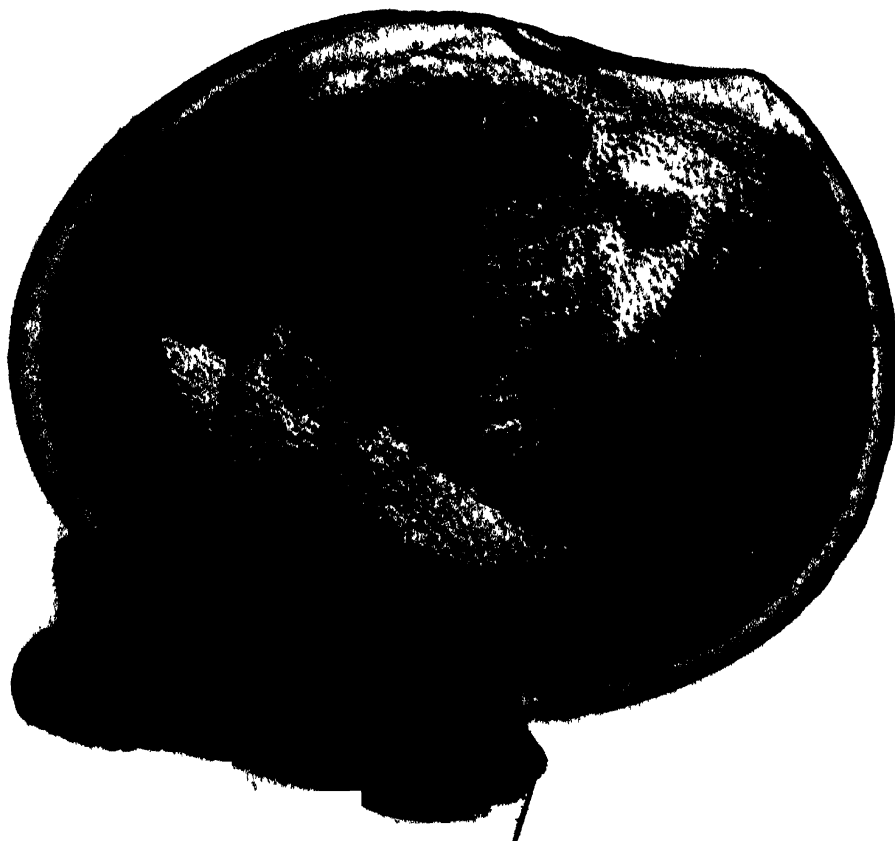
Fig.



Fig. 4



Fig. 7







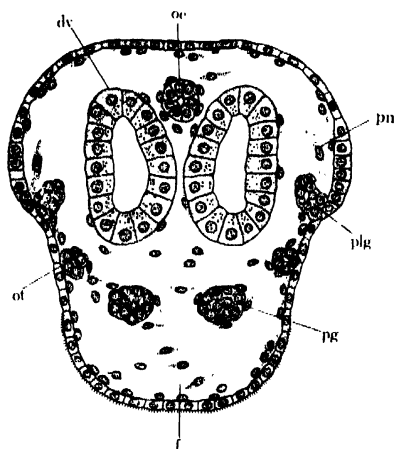


Fig. 8a

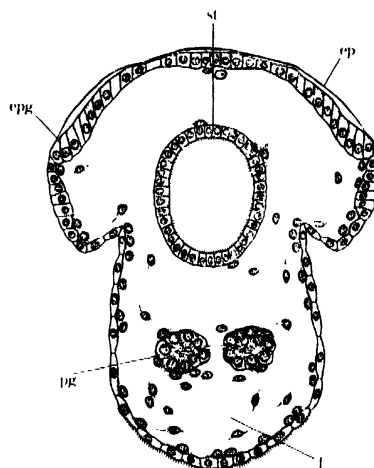


Fig. 8b

Fig. 8c

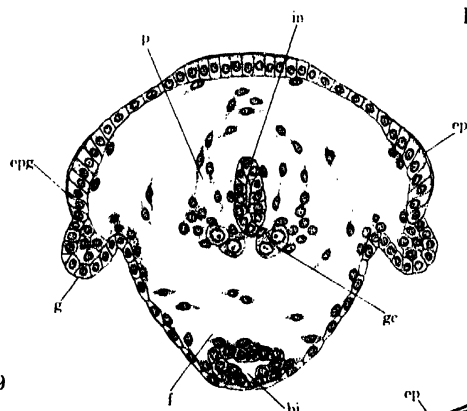
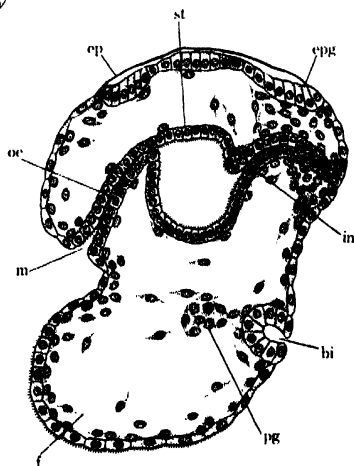
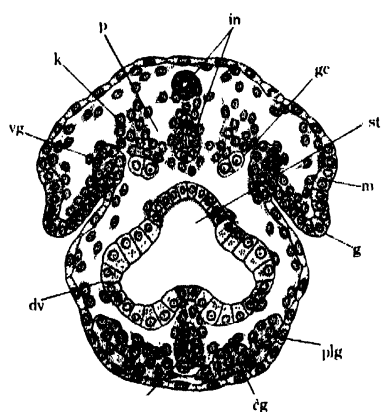


Fig. 9

Fig. 10





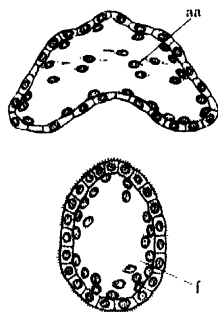


Fig. 11 a

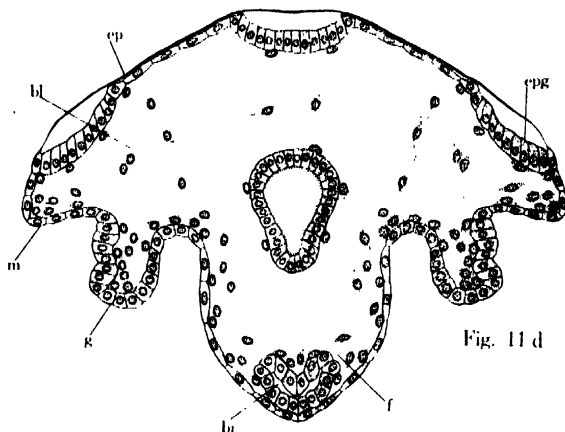


Fig. 11 d

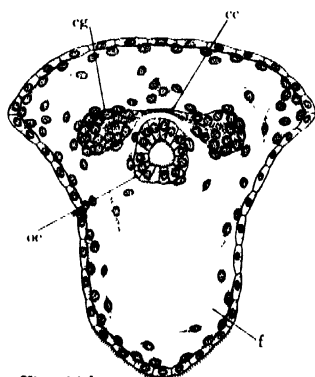


Fig. 11 b

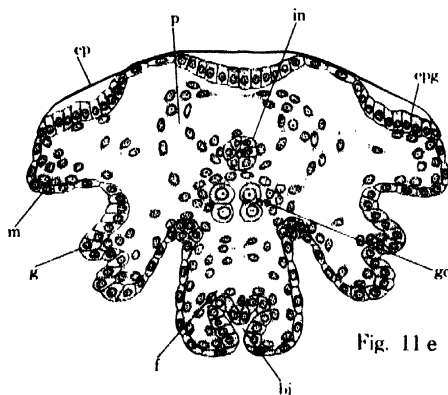


Fig. 11 e

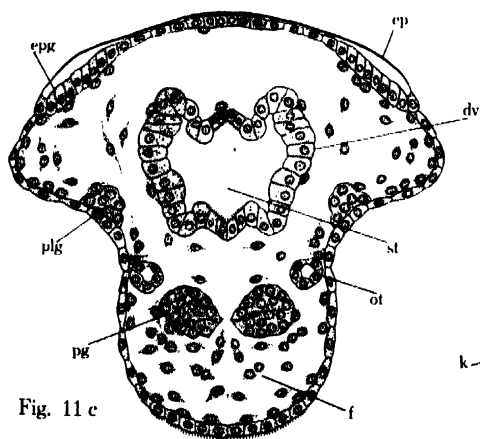


Fig. 11 c

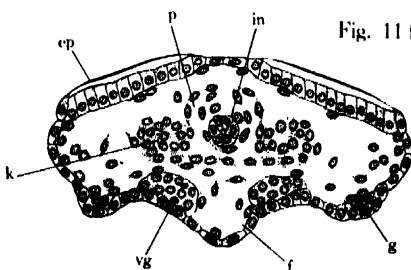


Fig. 11 f



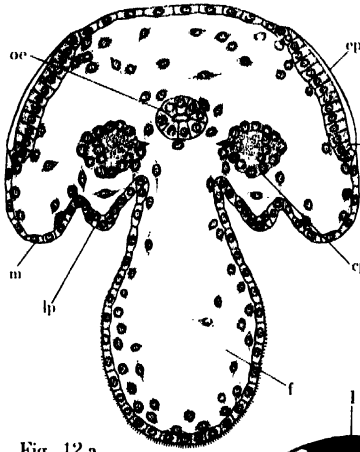


Fig. 12 a

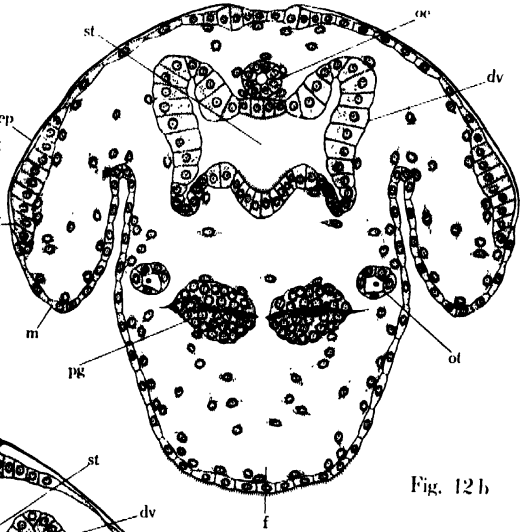


Fig. 12 b

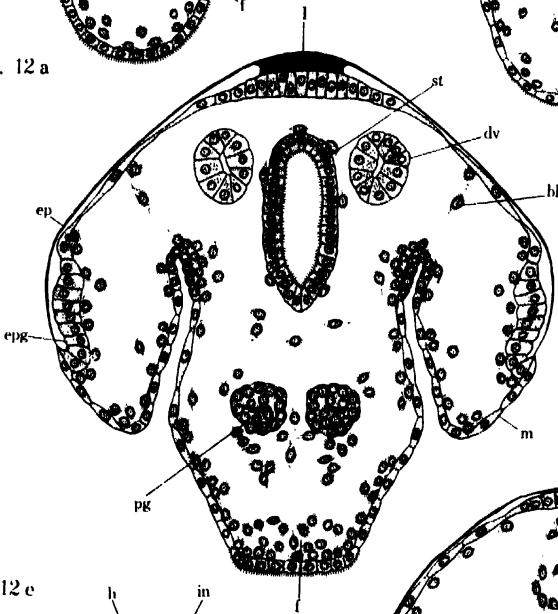


Fig. 12 c

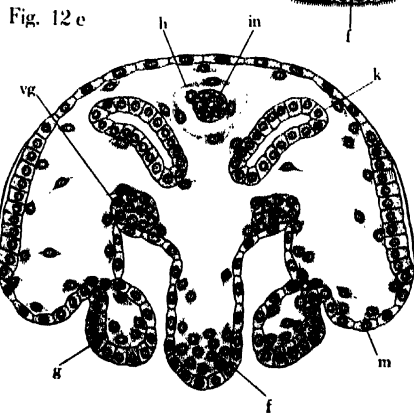


Fig. 12 e

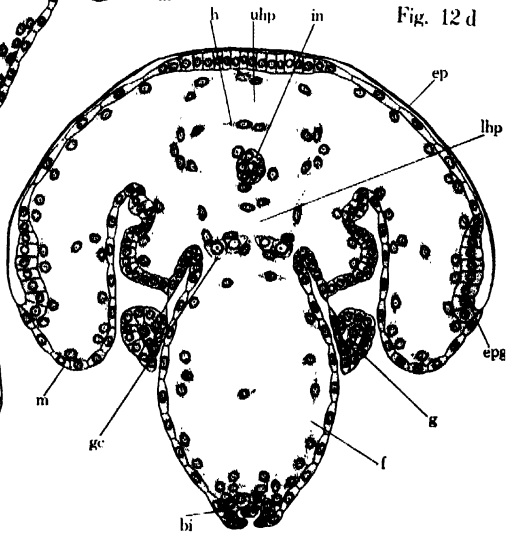


Fig. 12 d



# SEXUALITY OF RELATIVE GROWTH IN THE FRESH-WATER MUSSEL, *INVERSIDENS JAPONENSIS* (LEA)

By

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(With two figures)

(Received September 14, 1937)

Sexual dimorphism of bivalve shells is found merely in a few species. Among the species of the families, *Unionidae* and *Carditidae*, there are some particular genera, e. g. *Truncilla*, *Thecalia* and *Milneria*, having remarkable sexual characteristics (HAAS, 1929-1931)<sup>1)</sup>. Differences in the course of the growth of both sexes have been found in some fresh-water mussels (CHAMBERLAIN, 1930)<sup>2)</sup>. WEYMOUTH and McMILLIN (1930)<sup>3)</sup> have described sexual differences in the growth curve of *Siliqua patula*. They have, however, doubted the significance of the sexual differences because of their inadequate collection of the specimens for this study. The fresh-water mussel, *Inversidens japonensis*, has no very remarkable characteristics of sexual dimorphism, but from the point of view of relative growth, it has been found that the growth gradients of the two sexes of this species differ in quantity, respectively. This fact will be explained in the following pages.

Before proceeding further, the writer wishes to express his sincere thanks to Prof. E. NOMURA for his kind guidance, and also to Mr. SITIHEI NOMURA for the identification of the specimens of the species.

## MATERIAL AND METHOD

The materials were collected on May 5, 1936, in an irrigation channel at Masuda near Sendai, where there is always running water, the soil, at the depth in which the mussel lives, is muddy and red, and water-plants

<sup>1)</sup> HAAS, F. 1929-1931. Bivalvia. 173-178. Bronns Klass. u. Ord. d. Tierreichs. III. Abt. Leipzig.

<sup>2)</sup> CHAMBERLAIN, T. K. 1930. Annual Growth of Fresh-water Mussels. Bull. Bur. Fish. 46, 713-739.

<sup>3)</sup> WEYMOUTH, F. W. and McMILLIN, H. C. 1930. Relative Growth and Mortality of the Pacific Razor Clam (*Siliqua patula* DIXON) and their Bearing on the Commercial Fishery. Bull. Bur. Fish. 46, 543-567.



grow thick. In the stream in which the material species, *Inversidens japonensis* grows, the other mussel, *Anodonta japonica* CLESSIN was also found, but fewer in number than the former. The mussels were found between the roots of water-plants. 212 specimens were obtained, all of which were sexually mature, i. e. the ripe ova and the active spermatozoa were microscopically observed in every specimen. Among them 110 were males and the rest, 102, were females.

The same method of measurements, as described in the present writer's previous paper (1936)<sup>4)</sup> on the relative growth of the bivalves, *Cyclina*, *Gomphina*, etc., was applied in this investigation. That is, the distances between the umbo (the initial point of shell-development) and the various points in the shell margin, the distances between the points next to each other, and the length ( $L$ ), height ( $H$ ) and depth ( $D$ ) were measured. The dimensional expression in this paper is subject to the notations in Fig. 1.

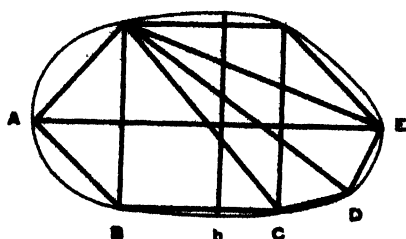


Fig. 1. Diagrammatic representation of points, where the measurements were taken.  $U$  the initial point of development,  $A$  anterior end,  $E$  posterior end,  $D$  angular end at postero-ventral part,  $F$  ligamental end,  $AE$  length,  $h$  height,  $UB$  and  $FC$  are perpendicular to  $AE$ . Thick lines show the dimensions measured.

The angles contained between points next to each other at the umbo were determined by calculation, with the following formula,

$$\sin C = 2\sqrt{s(s-a)(s-b)(s-c)}/ab$$

where  $a$ ,  $b$ , and  $c$  are the respective sides of a triangle  $ABC$ , and  $s = \frac{1}{2}(a+b+c)$ .

Up to the present time, the allometrical equation,  $y = bx^{\alpha}$ , has been applied to the study of the relative growth, where  $y$  is a dimension to be compared with the standard dimension  $x$ ,  $\alpha$  is the *equilibrium constant* of relative growth and  $b$  the *initial index*<sup>5)</sup>. Strictly speaking, this formula is merely an approximation

reasonable enough to be applied to a short range of  $x^0$ . But in the present case, the application of this formula has been possible to all the

<sup>4)</sup> HAMAI, I. 1936. Relative Growth in Some Bivalves. Sci. Repts. Tôhoku Imp. Univ. Biol. 10, 753-765.

<sup>5)</sup> This terminology and the notations are subject to HUXLEY and TEISSIER. HUXLEY, J. S. and TEISSIER, G. 1936. Terminology of Relative Growth. Nature, 137, 780-781.

<sup>6)</sup> HAMAI, I. 1937. Some Notes on Relative Growth with Special Reference to the Growth of Limpets. Sci. Repts. Tôhoku Imp. Univ. Biol. 12, 71-95.

specimens without serious error. Then, the length of the shell being settled as the standard, the various dimensions have been adopted and expressed as  $y$  in the formula: The growth gradient and other facts have been determined by the equilibrium constant calculated in this way with regard to every point in the shell margin.

## SEXUALITY IN GROWTH GRADIENT

The growth gradient in the shell margin is expressed by the distribution of the equilibrium constant  $\alpha$  in the relative growth of the dimensions,  $\overline{UA}$ ,  $\overline{UB}$ ,  $\overline{UC}$ , etc. (Table 1, Fig. 2). The sexuality in connexion

TABLE 1.

Dimension	$\alpha$		$\frac{\alpha \uparrow - \alpha \downarrow}{\text{P.E.}}$	$b$	
	$\uparrow$	$\downarrow$		$\uparrow$	$\downarrow$
$\overline{UA}$	$0.885 \pm 0.011$	$0.913 \pm 0.010$	1.9	0.4124	0.4056
$\overline{UB}$	$0.911 \pm 0.006$	$0.936 \pm 0.007$	2.8	0.5844	0.5739
$\overline{UC}$	$0.978 \pm 0.004$	$0.954 \pm 0.005$	4.0	0.7497	0.7745
$\overline{UD}$	$1.045 \pm 0.005$	$0.959 \pm 0.003$	14.3	0.7817	0.8641
$\overline{UE}$	$1.024 \pm 0.004$	$0.996 \pm 0.004$	4.7	0.7821	0.8003
$\overline{UF}$	$1.088 \pm 0.011$	$1.031 \pm 0.009$	3.8	0.4558	0.4863

with the growth gradient is probably recognizable in the margin posterior to point C. The general forms of the gradients are those which slope from the posterior to the anterior part. The most probable difference of sex within the growth gradients appears at point D, and it is the most significant. Generally, the gradient of the male is steeper than that of the female, and, in the posterior part, the values of

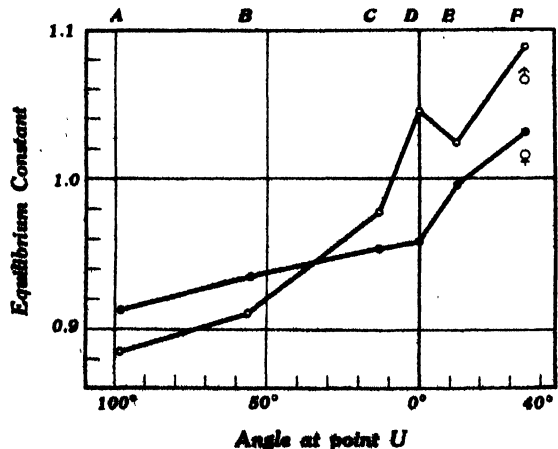


Fig. 2. The growth gradient in the shell margin in both sexes of *Inversidens japonensis*. Point D is taken as the standard.

$\alpha$  in the male are greater than those in the female.

When the three different heights, viz.  $\overline{UB}$ ,  $H$ , and  $\overline{FC}$ , are considered, a sort of growth gradient can be supposed to exist. But the sexual

TABLE 2.

Dimension	$\alpha$		$\frac{\alpha_{\text{♂}} - \alpha_{\text{♀}}}{\text{P.E.}}$	$b$	
	♂	♀		♂	♀
$\overline{UB}$	$0.911 \pm 0.006$	$0.936 \pm 0.007$	2.8	0.5844	0.5739
$H$	$0.913 \pm 0.006$	$0.912 \pm 0.007$	0.1	0.7002	0.6988
$\overline{FC}$	$0.829 \pm 0.008$	$0.837 \pm 0.007$	0.7	0.6765	0.6661

differences in these gradients are insignificant, and they slope from the anterior to the posterior part in both sexes (Table 2).

#### RELATIVE POSITION OF THE POINTS MEASURED

The relative position among the points in the shell margin is expressed by the angle which is contained by them at point  $U$ . The change in this angle, according to size or age, is very small, and is, therefore, negligible for the study of the growth gradient in the shell margin of this species. It is, then, necessary to consider that every point in the shell margin grows and develops radially in a straight line from the initial point  $U$ . Thus, the growth gradients mentioned above become accurate and significant. The angles expressing the relative position of the points are shown in Table 3.

TABLE 3.

Angle	Mean of angle		Difference
	♂	♀	
$\angle AUB$	$42^{\circ}23' \pm 7'$	$43^{\circ} 8' \pm 11'$	$45' \pm 13'$
$\angle BUC$	$42^{\circ}58' \pm 7'$	$42^{\circ} 5' \pm 8'$	$53' \pm 11'$
$\angle CUD$	$13^{\circ}12' \pm 8'$	$13^{\circ} 8' \pm 7'$	$4' \pm 11'$
$\angle DUE$	$12^{\circ}19' \pm 5'$	$12^{\circ}22' \pm 5'$	$3' \pm 7'$
$\angle EUF$	$22^{\circ}40' \pm 7'$	$22^{\circ}39' \pm 8'$	$1' \pm 11'$

The sexual differences indicated by these angles are generally insignificant, and the angles are probably equal in the two sexes. But  $\angle AUB$  and  $\angle BUC$  seem to be slightly different according to the sex. However,

it is merely assumed that the relative position of point  $B$ , and accordingly the relative position of  $\overline{UB}$ , is different, because the sum of these angles is probably equal in both sexes, and the relative position of this point lies more anteriorly in the male than that in the female. This fact may be correlated with the reversal of the values of  $\alpha$  between the two sexes, a point between  $B$  and  $C$  being a point of equal value: the values of the male are smaller in the anterior part, and greater in the posterior part, than those of the female.

## DISCUSSION

GRIER (*vide* HAAS<sup>1)</sup>) has studied the sexual dimorphism and found the following results: In several species, the shells of males are found higher and deeper than those of females, and the former are, therefore, relatively shorter than the latter. In other species the reverse is found. Males of some species are relatively larger in the posterior part of the shell than in the anterior part. In these species, as found by GRIER, the posterior

TABLE 4.

Species	$\frac{\text{Anterior end}}{\text{Posterior end}}$	
	♂	♀
<i>Anodonta grandis</i>	0.406	0.387
<i>Symphynota costata</i>	0.370	0.344
<i>Fusconaja flava</i>	0.306	0.299
<i>Pleurobema coccineum obliquum</i>	0.275	0.184
<i>Amblema plicata</i>	0.233	0.246
<i>Elliptio dilatatus</i>	0.255	0.273
<i>Eurynia recta</i>	0.259	0.288
<i>Anodontoides ferussacianus</i>	0.329	0.401
<i>Lampsilis luteola</i>	0.354	0.360
<i>Paraptera fragilis</i>	0.362	0.365
<i>Proptera alata</i>	0.412	0.461
<i>Lampsilis ovata ventricosa</i>	0.421	0.451

part of the hinge was better developed in the male, and the anterior part in the female. From the table summarized by HAAS<sup>1)</sup>, the ratios between the anterior and the posterior end are given (Table 4). It is found that

<sup>1)</sup> HAAS, F. 1929-1931. *loc. cit.* p. 178.

the development of the anterior or posterior part of the shells varies in different degrees. Table 4 shows the statistical results, but these cannot be recognized during the whole process of growth, for the growth is in general allometrical.

HAYASHI (1935)<sup>7)</sup> has observed that in *Anodonta woodiana* the index  $D/H.L$  decreases hyperbolically with the increase of length, and that it is always larger in the female than in the male. For the study of the form of shells, such a dynamical test is necessarily important rather than the values as shown in Table 4. From this point of view, the degree of development is given by the equilibrium constant, which is the ratio of the specific growth rates of the two parts in question. The relation of the anterior part ( $\overline{UA}$ ) to the posterior ( $\overline{UE}$ ) in the *Inversidens japonensis* is as follows:

$$\alpha = 0.864 \quad \text{in male}; \quad \alpha = 0.916 \quad \text{in female};$$

i. e. in the female the anterior part develops more than in the male. But the depth shows probably no sexual difference, as  $\alpha = 0.891 \pm 0.010$  in the male and  $= 0.916 \pm 0.009$  in the female.

As it has been shown by CHAMBERLAIN<sup>2)</sup> that the growth process is different in the male and female of *Lampsilis anodontoides*, and the differences are correlated with the changes in the contour of the shell accompanying sexual maturity, in the case of *Inversidens japonensis* also the same conclusion is drawn from the differences in the growth gradients in the two sexes, viz. every part of the shell margin, especially the posterior margin, shows different specific rates of growth, and therefore, the contour of the shell of the two sexes changes owing to the different modes of growth. In general, the sexuality of bivalves should be established in the process of growth.

The present writer<sup>4)</sup> has previously shown, by comparing the rates of the anterior and posterior ends, that there are two types of shell-growth, one of which has a higher rate of relative growth in the anterior end, viz. *Cyclina sinensis*, and the other in the posterior end viz. *Gomphina melanaegis*. *Inversidens japonensis* is found to belong to the latter type from its gradient of relative growth in the shell margin, by which the sexuality is disclosed in detail. The sexual differences in the gradient in the posterior part, from points  $D$  to  $F$ , may suggest an interesting implication, i. e. because in this part the siphons open, the problem may

<sup>2)</sup> CHAMBERLAIN, T. K. 1930. *loc. cit.*

<sup>4)</sup> HAMAI, I. 1936. *loc. cit.*

<sup>7)</sup> HAYASHI, K. 1935. On the Convexity of the Shell of *Anodonta*. *Venus*, 5, 23-25.

arise as to whether a correlation between the growth of the shell margin and the morphological and functional changes of siphons and of other organs in this region exists, and, consequently, whether there is any actual sexuality in the siphons and the other organs or not.

#### CONCLUSIONS

From the point of view of relative growth, the sexuality of *Inversidens japonensis* has been studied, and the following conclusions have been arrived at.

- 1) The growth gradient which slopes from posterior to anterior in the shell margin is steeper in the male (1.088-0.885) than in the female (1.031-0.913).
- 2) In the posterior part of the shell, the values of the equilibrium constant are higher in the former than in the latter.



# ENTWICKLUNG DER FORTPFLANZUNGSORGANE UND KEIMUNGSGESCHICHTE VON *DESMARESTIA* *VIRIDIS* (MÜLL.) LAMOUR<sup>1)</sup>

VON

KÔGORÔ ABE

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(Mit Tafel XXXIX und sechs Textfiguren)

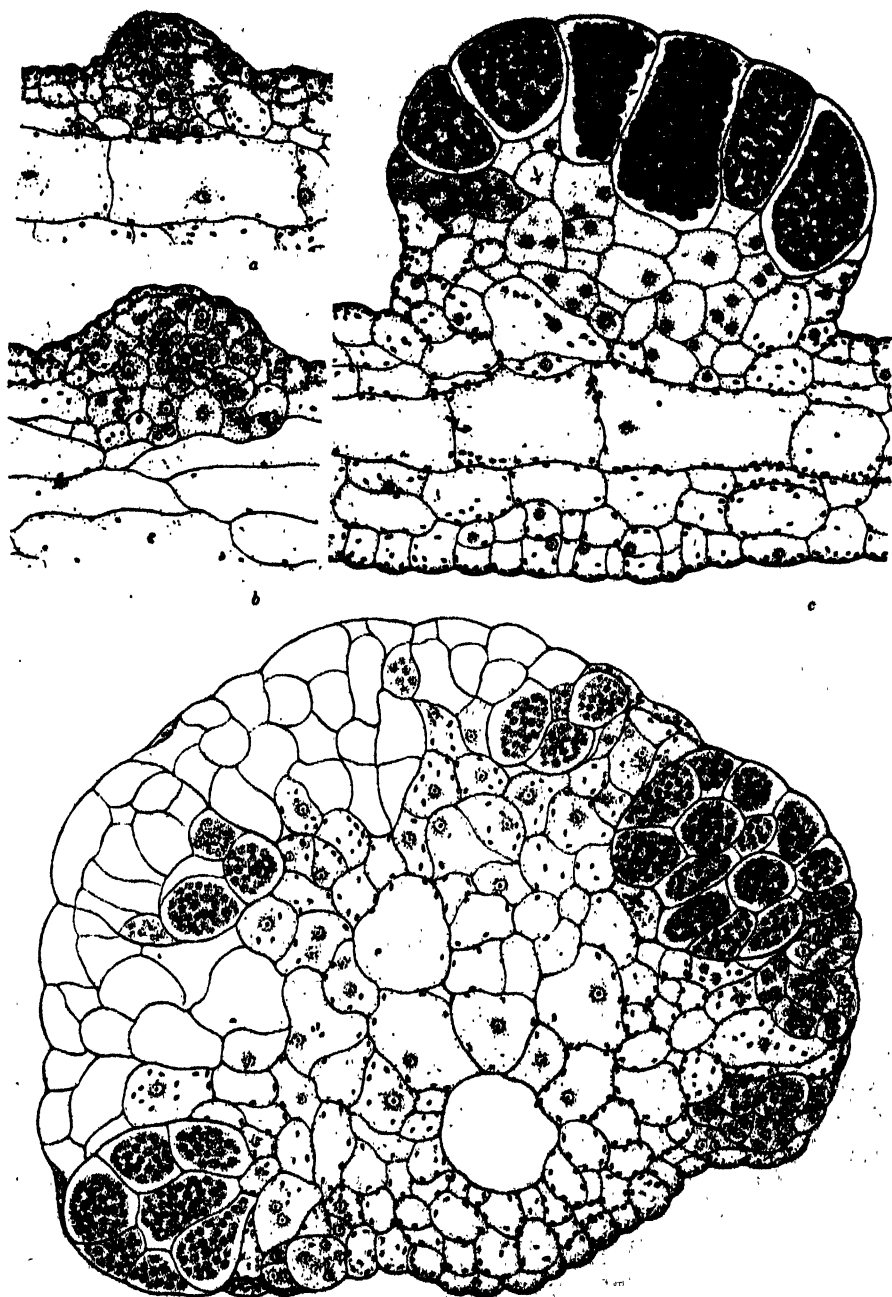
(Eingegangen am 14. Oktober 1937)

Über die Lebensgeschichte von *Desmarestia aculeata* (L.) LAMOUR. machte SCHREIBER 1932 eine auffallende Mitteilung. Nach ihm zeigt diese Braunalge einen regelmässigen Generationswechsel zwischen einer zwerghaften geschlechtlichen und einer stattlichen ungeschlechtlichen Generation, wie bei den Familien Laminariaceen und Chordaceen. Er führte seine Beobachtung auf in Kultur befindlichen Pflänzchen aus. Die in unilokulären Sporangien gebildeten Schwärmer keimten ungeschlechtlich und wuchsen zu monosiphonen männlichen oder weiblichen Gametophyten aus. Auch die Zygotenkeimung und Weiterentwicklung bis zur jungen Sporophytenpflanze wurden von ihm ausführlich verfolgt. Um diese merkwürdige Tatsache noch bei anderen Spezies dieser Familie festzustellen, weilte ich im Frühling letzten Jahres etwa zwei Monate in der biologischen Station zu Asamushi. Als Material für die Untersuchung wählte ich *Desmarestia viridis* (MÜLL.) LAMOUR. In dieser Gegend findet man diese Alge nicht selten. Es war aber nicht so leicht, Fortpflanzungsorgane tragende Pflanzen zu finden. Nach längerer erfolgloser Mühe gelang es mir doch erst am 18 April und nachher noch einige Male eine Anzahl der voll ausgereiften Pflanzen zu gewinnen. Mit diesen Materialien habe ich einige vorläufige Untersuchungen gemacht. Im Winter dieses Jahres begab ich mich wieder nach Asamushi. Mitte März konnte ich zum ersten Male ein lang gesuchtes, unreife Fortpflanzungsorgane tragendes Individuum gewinnen. Diese Pflanze lieferte mir ein fast genügendes Material für die Untersuchung über die meiotischen Teilungen im Sporangium.

Über die Fortpflanzungsorgane dieser Spezies haben schon früher THURET und BORNET (Et. Phyc. p. 16, zitiert nach JOHNSON 1891) kurz mitgeteilt. Aber ihre Beschreibung ist ganz unvollständig. So möchte

<sup>1)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-Ken, No. 146.





Textfig. 1. Sorus von *Desmarestia viridis* in verschiedenen Entwicklungsstadien.  
Erklärung im Text. Vergr. 280.

ich unten zunächst hierüber etwas genauer mitteilen. Es ist schwer auf Handschnitten die feinere Struktur dieser Organe zu erkennen. Meine diesbezügliche Beobachtung wurde daher ausschliesslich auf Mikrotomschnitten durchgeführt. Das Material wurde mit einer Lösung fixiert, welche ich bei der Untersuchung über die Mitosen im Antheridium von *Sargassum confusum* (ABE 1933) und den Kernphasenwechsel von *Heterochordaria abietina* (ABE 1936) verwandt hatte. Die  $9\mu$  dick geschnittenen Paraffinschnitte wurden mit HEIDENHAINS Eisenalaunhämatoxylin gefärbt.

Im ersten Stadium der Entwicklung der Fortpflanzungsorgane sieht man kleine Höcker an verschiedenen Stellen des Thallus. Sie entstehen durch Teilungen und darauffolgendes Wachstum der Rindenzellen. Dabei bleiben aber die oberst liegenden Zellen ganz flach (Textfigg. 1, a-b). Sie



Textfig. 2. Sorus von *Desmarestia viridis*. Vergr. 66.

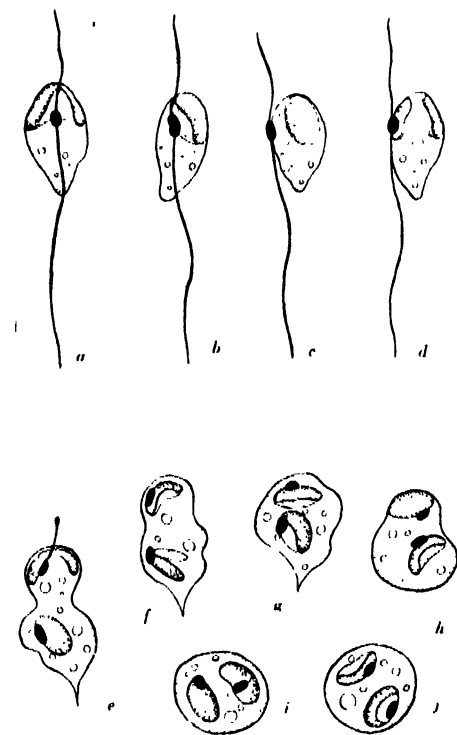
sind steril und werden später durch das Wachstum der inneren fertilen Zellen gesprengt und vernichtet (Textfig. 1, c). Diese fertilen Zellen reihen sich in ein bis mehreren Schichten. Die meiotischen Teilungen in diesen Zellen vollziehen sich in folgender Weise:

Taf. XXXIX, Fig. 1 zeigt den sich im vollständigen Ruhestadium befindlichen Kern solcher Zellen. Ein Synapsisstadium kann man in Figg. 3-4 ansehen. Das Spirem- und Diakinesestadium folgen darauf (Figg. 5-9). Bei solchen Stadien kann man feststellen, dass die Zahl der Chromosomen mit grosser Wahrscheinlichkeit 22 beträgt. Die vollständige Metaphase erfolgt nach Auflösung der Kernmembran. Fig. 10 gibt dasselbe Stadium in Polansicht wieder, wobei sich auch die etwa 22 reduzierten Chromosomen zählen lassen. In der Seitenansicht der Spindel

ist ein zentrosomähnliches Körperchen in jedem Pole sichtbar (Fig. 12). In der Telophase berühren sich die beiden Tochterkerne eng aneinander (Fig. 13). Nach kurzer Pause beginnt die homöotype Teilung (Fig. 14-15). Nach dieser Teilung entstehen natürlich vier Kerne (Fig. 16), die dann noch weitere simultane Teilungen ausführen. Inzwischen vergrößert

sich die Zelle allmählich (Textfig. 1, c-d). Und am Ende teilt sich der Protoplast in viele winzige, mit je einem Kerne ausgestattete Zellen, welche sich später zu Schwärmer entwickeln. Der aus solchen unilokulären Sporangien bestehende Sorus sitzt gewöhnlich am Ende eines kurzen stumpfährlichen Stiels (Textfig. 1, c). Textfigg. 2, a b zeigen uns das Ansehen von diesem Gebilde. Es ist auffallend, dass bei den bisher untersuchten Spezies von *Desmarestia* der Sorus keine merkliche Erhöhung an der Oberfläche des Thallus macht.

So weit über die Entwicklung der Fortpflanzungsorgane. Nächst werde ich einiges über die Kulturversuche mitteilen. Wenn man reifes, vorher mit filtriertem Meereswasser ausgespültes Material in ein grosses Glasgefäß mit Meereswasser bringt, so bekommt man nach einiger Zeit

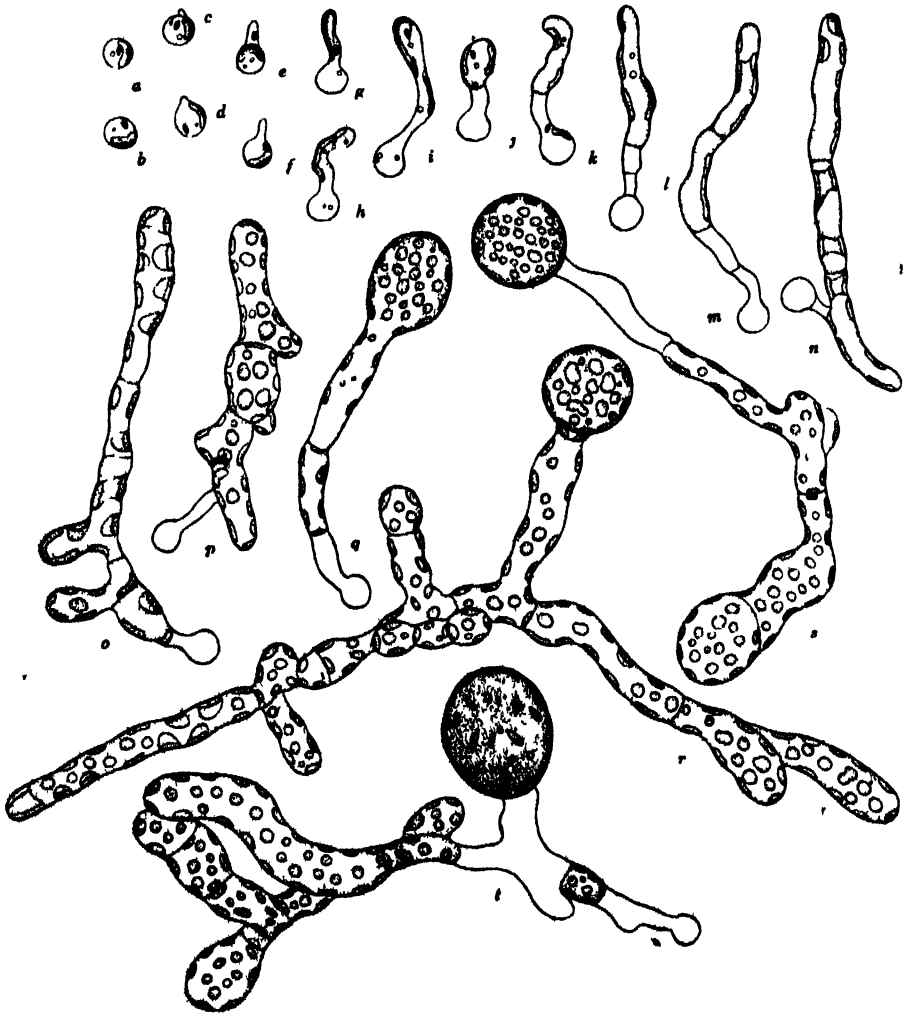


Textfig. 3. *D. viridis*. Schwärmer und ihre Kopulation. a-d Schwärmer aus unilokulären Sporangien. e-j aufeinanderfolgende Stadien der Kopulation. Vergr. 1430.

eine Menge von Schwärmern. Sie bewegen sich lebhaft und zeigen gewöhnlich negative Phototaxis. Die Messung der Schwärmer ergab etwa 8-10  $\mu$  lang und 5-6  $\mu$  breit. Die Schwärmer besitzen je ein platten- oder kragenförmiges Chromatophor und einen roten Augenfleck. Die beiden Geisseln entspringen seitlich in der Mitte des Augenflecks, wie bei den anderen Braunalgen (Textfigg. 3, a-d)<sup>2)</sup>. Kopulation zwischen den Schwärmern

<sup>2)</sup> SCHREIBERS Zeichnungen der Schwärmer sehen etwas anders aus.

konnte SCHREIBER niemals bei seiner Pflanze beobachten, auch dann nicht, als Material von verschiedenen Pflanzen zu den Versuchen verwandt wurde. Aber bei meiner Spezies verläuft die Kopulation manchmal zwischen den Schwärmern desselben Individuums (Textfigg. 3, e-j)<sup>\*)</sup>. Beispiele der



Textfig. 4. Keimlinge der Schwärmer von *D. viridis*. a-b Embryospore, c-f 1 Tag alt. g-i 2 Tage alt. j-k 4 Tage alt. l-n 8-10 Tage alt. o-p 15 Tage alt. q-t Weibliche Geschlechtsorgane tragende Pflänzchen. Vergr. 450.

<sup>\*)</sup> Die Schwärmer und ihr Kopulationsverlauf wurden bei den mit Osmiumsäure-Dampf fixierten Materialien abgebildet.

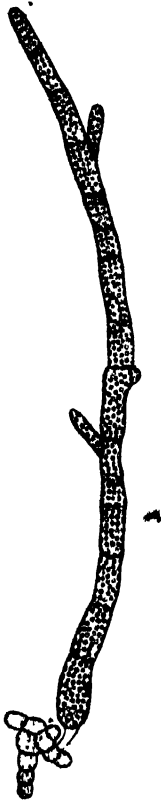
Kopulation der in unilokulären Sporangien gebildeten Schwärmer wurden jüngst bei anderen Algen von verschiedenen Forschern gefunden, nämlich: *Pylaiella littoralis* (KNIGHT, 1923), *Ectocarpus siliculosus* (KNIGHT, 1929. SCHUSSNIG, 1934), *Sphacelaria bipinnata* (CLINT, 1927), *Heterochordaria abietina* (ABE, 1935). Kulturversuche der Schwärmer und der durch Kopulation der Schwärmer entstandenen Zygoten geschah auf den Objektträgern, gehalten in einem grossen Glasgefässe, welches fortwährend ringsum durch das kalte Leitungswasser gespült wurde. Als Nährlösung benutzte ich hauptsächlich neuere SCHREIBERSche Flüssigkeit (1935), welche er bei seinem Kulturversuch von *Dictyota dichotoma* mit bestem Erfolg gebraucht hatte.

Nach etwa 24 bis 30 Stunden beginnen die Schwärmer zu keimen und entwickeln einen Keimschlauch (Textfigg. 4, *a-f*). Die Spitze des Keimschlauches nimmt dann keulenförmige Gestalt an (Textfigg. 4, *g-h*). Nach einigen Tagen erfährt das Chromatophor eine Zweiteilung und das Ende des Schlauches wird durch eine Querwand gegen die Basis abgegrenzt (Textfigg. 4, *i-k*). Die Vermehrungen der Chromatophoren und die Zellteilungen folgen dann aufeinander (Textfigg. 4, *l-p*) und fängt die Ausbildung der Geschlechtsorgane an. Eine Zelle des Keimlings schwillt zuerst an ihrem freien Ende keulenförmig an und ihr Protoplast tritt dann in einer sphärischen Form durch eine Öffnung an der Spitze nach aussen hervor. Diese sphärische Zelle bleibt in der Regel an der Mündung der leeren Hülle. Sie kann als Eizelle betrachtet werden (Textfigg. 4, *q-t*). Ausserdem findet man in den Kulturen nicht selten etwas anders aussehende, kleinere Chromatophoren besitzende Keimlinge. Allem Anschein nach mögen diese die männlichen Individuen sein. Die Antheridien konnte ich aber nicht mit Bestimmtheit konstatieren. Überhaupt sind die Bildungen der Geschlechtsorgane keinesweges an einem bestimmten Entwicklungszustand der Keimlinge gebunden; denn sie finden sich sowohl an wenigzelligen als auch vielzelligen Keimlingen vor. In weiteren Fortschritten der Kultur nach etwa einigen Monaten bemerken wir die vom Substrat mehr oder weniger aufrecht stehenden monosiphonen spiessförmigen Fäden. Ihre grossen Chromatophoren sind selbst unter schwächerer Vergrösserung deutlich erkennbar. Sie dürfte das Anfangsstadium der Sporophytengeneration sein (Textfig. 5).

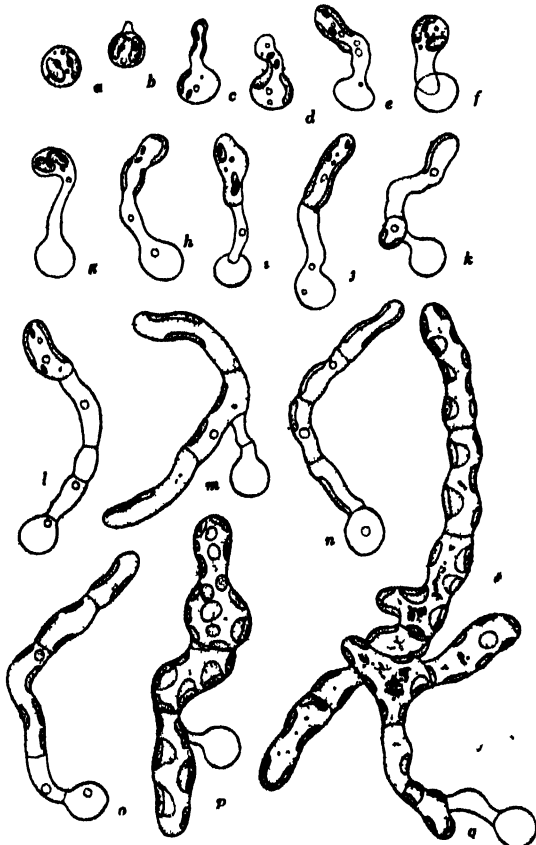
Die Keimung der durch die Kopulation der Schwärmer entstandenen Zygoten verläuft ganz wie die der Schwärmer (Textfigg. 6, *a-q*). Ihr weiteres Schicksal konnte noch nicht verfolgt werden.

Ungeachtet der Unvollständigkeit des vorliegenden Kulturversuches

scheint es mir wahrscheinlich, dass diese Spezies auch wie bei *Desmarestia aculeata* einen regelmässigen Generationswechsel zwischen einer geschlecht-



Textfig. 5. *D. viridis*.  
Anfangsstadium der Sporophytengeneration. Etwa  
53 Tage alt. Vergr. 190.



Textfig. 6. *D. viridis*. Keimlinge der durch Kopulation  
der Schwärmer entstandenen Zygoten. Vergr. 700.

lichen haploiden und einer ungeschlechtlichen diploiden Generation aufzeigen darf.

#### ZUSAMMENFASSUNG.

Der aus unilokulären Sporangien bestehende Sorus von *Desmarestia viridis* macht eine merkliche Erhöhung an der Oberfläche des Thallus. Die zwei vorersten Kernteilungen im Sporangium sind die Reduktionsteilungen, wobei sich die etwa 22 reduzierten Chromosomen zählen lassen.

Die aus Sporangien hervor tretenden Schwärmer keimen meistens ungeschlechtlich, aber manchmal auch nach Kopulation. Die Keimlingen sind sehr ähnlich denjenigen von *Desmarestia aculeata*.

In älteren Kulturen finden wir die spießförmigen, ganz anders aussehenden, vom Substrat aufrecht stehenden Keimpflänzchen. — Wahrscheinlich das Anfangsstadium der Sporophyten.

Meinem hochverehrten Lehrer, Herrn Prof. Dr. M. TAHARA, möchte ich an dieser Stelle für die reiche Anregung und das freundliche Interesse bei Ausführung dieser Arbeit meinen herzlichsten Dank aussprechen.

#### LITERATURVERZEICHNIS.

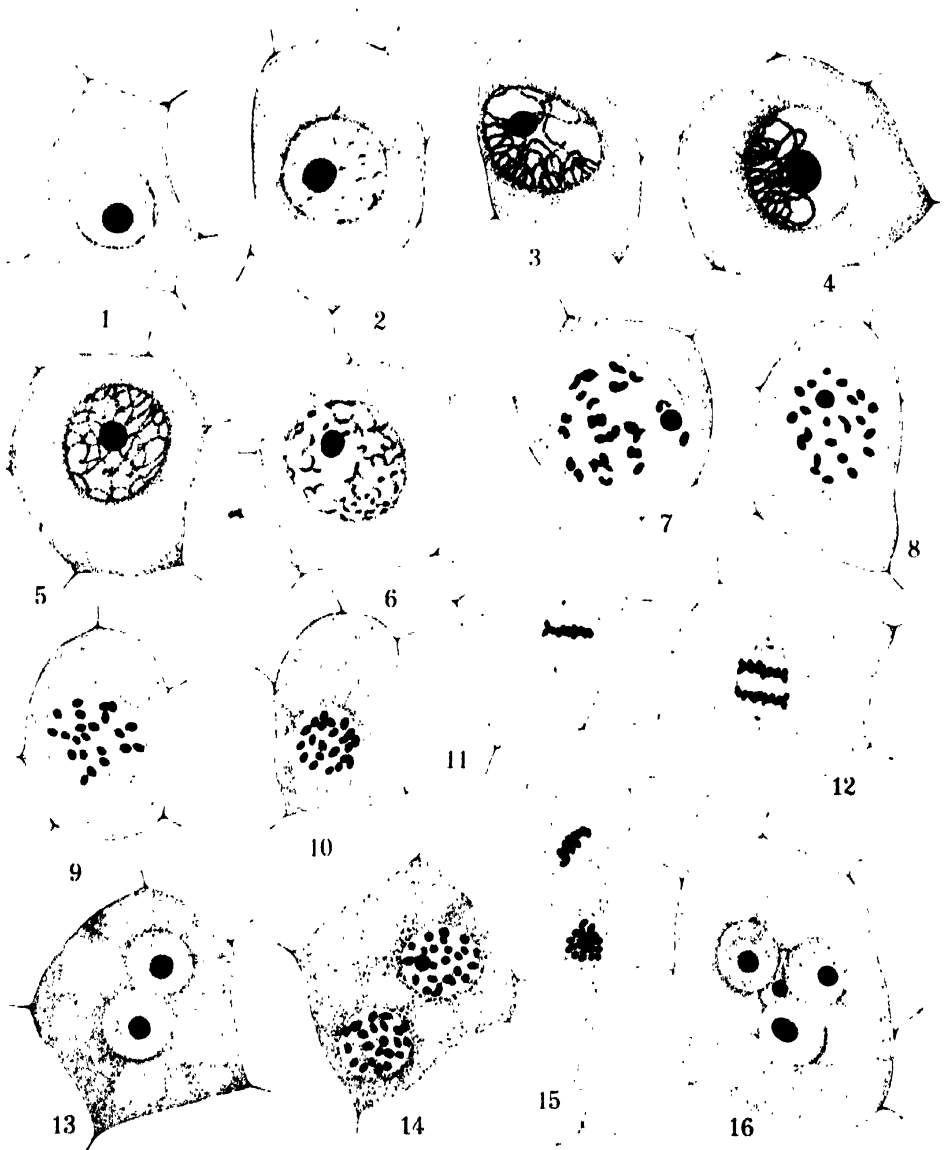
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#### TAFELERKLÄRUNG.

Alle Figuren wurden mit Hilfe eines Anstischen Zeichenapparatus gezeichnet, unter Benutzung des Zeisschen Objektiv, Ölimmersion 1/12 und des Zeisschen Okular  $\times 17$ . Vergr.

#### TAFEL XXXIX.

Fig. 1. Ruhestadium. Fig. 2. Früheste Prophase. Figg. 3–4. Synapsis, Figg. 5–9. Diakinesis. Fig. 10. Vollständige Metaphase in Polansicht. Fig. 11. Dieselbe in Seitenansicht. Fig. 12. Anaphase. Fig. 13. Telophase. Figg. 14–15. Homöotypen Teilungen. Fig. 16. Vierkerniges Stadium.







# GLYCOGENOLYSIS IN THE NERVE CELLS AFTER SECTION OF THEIR AXONES, WITH REFERENCE TO CHROMATOLYSIS

By

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(With Plate XL)

(Received October 20, 1937)

## INTRODUCTION

In the previous paper (1937) I reported that the glycogen in the nerve cells of the horse is contained in the Nissl's bodies, and therefore, as far as the Nissl staining is possible the glycogen granules of the same form and arrangement as the Nissl's bodies appear. KIMURA (1934) also reported, though without any decisive evidence, that glycogen is found in the Nissl's bodies of the ganglion cells of the aves and mammals. On the other hand, it has been generally known that the so-called chromatolysis, the disappearance of the Nissl's bodies, wholly or in part, occurs in the nerve cells under various pathological conditions, accordingly the changes of glycogen granules in such cells as undergoing chromatolysis is of great interest.

In the present investigation I have dealt with the morphological changes of glycogen granules in the nerve cells after section of their axones, with reference to the relation of it to the chromatolysis, as the second step of investigation concerning the relation between the glycogen and the Nissl's bodies.

## MATERIALS AND METHODS

All the materials were taken from guinea pigs. Six healthy adults were selected and the neurectomy of the nervous branches arising from the brachial plexus going to the anterior limb was made. The operation was performed as follows: Clean and sterilised instruments were used. The animals were lightly etherized, and the skin and the superficial and deep pectoral muscles on the right side were incised without haemorrhage. All the nerve branches, N. suprascapularis, Nn. subscapulares, Nn. pectorales, N. musculocutaneus, N. medianus, N. ulnaris, N. radialis and N.

axialis, that appeared through the incision, were cut off about 1.5 cm. long. The muscles and the skin were sewn. No specimen became septic after the operation.

At fixed intervals after the operation the animals were killed without anaesthesia by cutting the carotid vein, and the spinal ganglia and the spinal cord of the cervical region, from which the brachial plexus arises, were obtained and used for the histochemical analysis of the relation between the glycogenolysis and the chromatolysis. The ganglia of the left side, the nerves arising from which had not been injured, were also used for control.

Alcohol-formalin saturated with sodium acetate was used as fixing fluid. All the materials were embedded in celloidin and sectioned 15  $\mu$  thick.

The following stains were used: Methylene blue or toluidin blue for Nissl's bodies, Best's carmin fluid for glycogen.

## RESULTS

### *1. Result Obtained for the Morphological Relation between the Nissl's Bodies and the Glycogen Granules under the Normal Conditions.*

I have preliminarily studied the normal morphology of the glycogen granules in the nerve cells of the guinea pig, with reference to the relation of it to the Nissl's bodies. The observation is as follows:

In the large motor cells in the anterior horn of the spinal cord the glycogen granules are of angular form of conspicuous size, which often exceed in dimension the large nucleolus of the nerve cell. They are to be found not only in the cell body, but also for a distance in the dendrites, where they have a more elongated, spindle shape. The neurite and its cone of origin are free from them. Sensory cells in the posterior horn of the spinal cord contain a very few large spindle shaped granules. In the cells of the spinal ganglion, the glycogen granules are in an irregular roundish form of large size and closely packed in the body of the cell. It was also found, by the method stated in the previous paper (1937), that the glycogen granules just stated are morphologically the same as the Nissl's bodies (Figs. 1 and 2), showing that the glycogen is contained in the nerve cells at least as one of the elements of the Nissl's bodies. This confirms the result obtained for the glycogen in the nerve cells of the horse (1937).

## 2. *Result Obtained for the Morphological Relation between the Glycogen and the Nissl's Bodies after Section of the Axone.*

To obtain further data concerning the evidences for the presence of glycogen in the Nissl's bodies the morphological changes of glycogen granules in the nerve cells after section of the axone, with reference to chromatolysis was investigated. The observation is as follows:

In the body of the spinal ganglion cell glycogenolysis was found, its degree increasing with the lapse of time after the operation. In about 7 days after neurectomy the glycogen granules decreased in size (Fig. 4). In some cells the change was most marked in the central portion, these glycogen granules in the periphery often remaining in the form of a ring. In from about 14 to 21 days after the operation the change reached its maximum, when they almost disappeared throughout the entire body of the cells (Fig. 6). Succeeding period showed the recovery, namely in about 28 days after the operation the reappearance of the granules was observed and until about 42 days their form and arrangement were found to be normal.

It was also found that some of the spinal ganglion cells remained without any degree of glycogenolysis. This shows that the axones of these cells were not sectioned, accordingly they were not affected by a lesion of the spinal nerve.

As regards the relation of glycogen to the Nissl's bodies, I have already stated (1937), that since the glycogen contained in the nerve cells is one of the elements of the Nissl's bodies, the disappearance of these bodies by the action of digestive enzymes or by a postmortem autolysis also shows the disappearance of glycogen. To determine whether or not the glycogenolysis that occurred in the nerve cells after section of the axone is also related to the disintegration of the Nissl's bodies, the sections from the neurectomized animals were used for both the Nissl staining and the glycogen staining by the method stated in the previous paper (TORYU, 1937). The microscopic examination was made on the section thus treated and the Nissl's bodies were morphologically compared with glycogen granules. The observation is as follows:

The disintegration, the so-called chromatolysis was found after the operation. Its degree increased with the increase of time up to about from 14 to 21 days, when the Nissl's bodies almost disappeared (Fig. 5); succeeding period showed the recovery, reaching the normal state in about 48 days after the operation.

It was found that the disintegration and the recovery of glycogen always agree with those of the Nissl's bodies above mentioned, namely with the decrease or increase of the Nissl's bodies the glycogen also decreased or increased with the same manner as the bodies, and finally when the bodies disappeared the glycogen also completely disappeared (Fig. 6), but when the bodies reappeared the glycogen also did so.

From the result above obtained it seems to me that since the glycogen contained in the nerve cells is one of the elements of the Nissl's bodies the chromatolysis is accompanied by glycogenolysis.

In the cells of the spinal cord after section of the axone chromatolysis was not found at all. In about from 7 to 48 days after the operation the cells were filled with the Nissl's bodies of normal form and arrangement, accordingly the glycogen also remained in normal state.

As to the degree of the reaction of the nerve cells to cutting their axones, it is generally thought that there is a difference in different types of the cells. It seems to me that the nerve cells in the spinal cord are much more resistant than the spinal ganglion cells from the fact that the chromatolysis or the glycogenolysis was not found in the former, but found in the latter as already mentioned.

#### SUMMARY

1. Glycogen granules in the nerve cells coincide morphologically with the Nissl's bodies; the Nissl's bodies can be stained with Best's carmin fluid.

2. Glycogenolysis occurs in the spinal ganglion cells after section of their axone; it reaches maximum in about from 14 to 21 days and recovery takes place within about 48 days after the operation.

3. The disintegration of the Nissl's bodies, the so-called chromatolysis is accompanied by glycogenolysis, but when the recovery of the Nissl's bodies takes place the glycogen granules of the same form and arrangement as the bodies reappear, showing that the glycogen in the nerve cells is contained in the Nissl's bodies.

4. The nerve cells of the spinal cord are not affected by the neur-ectomy in the present experiment; no chromatolysis occurs and accordingly the glycogen granules remain normal.

Before leaving the subject, I wish to express my hearty thanks to Dr. S. HATAI for his valuable suggestions and criticism throughout the entire course of this work. My thanks are also due to Dr. K. KIKUCHI who helped me with much kindness during this work.

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## EXPLANATION OF PLATE XL

- Fig. 1. Nissl's bodies in the spinal ganglion cells.  $\times 650$ .
- Fig. 2. Glycogen in the same cells as the above. (The Nissl's bodies were decolorized and then stained with Best's carmin fluid.)  $\times 650$ . (The form and arrangement of the glycogen granules are the same as those of the Nissl's bodies.)
- Fig. 3. Nissl's bodies in the spinal ganglion cells in 7 days after section of their axone.  $\times 650$ . (Nissl's bodies are decreased in size and amount, especially in the central portion of the cells.)
- Fig. 4. Glycogen in the same cells as the above.  $\times 650$ . (Glycogen also diminished in the same manner as the Nissl's bodies.)
- Fig. 5. Nissl's bodies in the spinal ganglion cells in 21 days after section of their axone.  $\times 650$ . (Nissl's bodies have almost completely disappeared.)
- Fig. 6. Glycogen in the same cells as the above.  $\times 650$ . (Glycogen has also completely disappeared.)



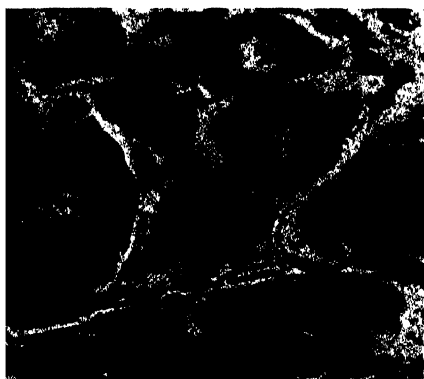


Fig. 1.

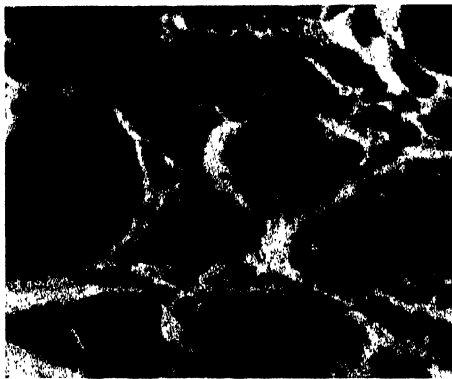


Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.





# THE SENSIBILITY OF THE BARBEL OF *UPENEUS SPILURUS* BLEEKER, WITH SOME NOTES ON THE SCHOOLING

By

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(With five figures)

(Received October 20, 1937)

Previously (SATÔ, '37 a, b), the importance of the barbels of *Upeneoides bensasi* in sensing food was described, and in the present work the same problem is directed to *Upeneus spilurus*. This goat-fish possesses two notable barbels at the chin and seems to be favorable for such a study.

This species, as *Upeneoides bensasi*, is found along the southern coast of Japan but differs in attaining a greater length (about 300 mm.) than the latter. The specimens used in the present work measured about 180 mm. in length and all were obtained from Simoda Bay.

At this place I wish to record my sincere gratitude to Prof. Dr. S. HATAI for this cordial guidance given me during the course of this study and also I wish to offer my thanks to Mr. TAKANAGA MITSUI for providing me with facilities during this investigation.

## EXPERIMENTS WITH NORMAL FISHES

In experimenting on the normal fish, I used a laboratory aquarium measuring 70 cm. in length, 41 cm. in breadth and 36 cm. in height. Its bottom and three sides were made of wood, while one side was of plate glass for the sake of observations. A layer of sand was spread on the bottom of the aquarium to immitate the natural habitats.

i. *The schooling behavior.* One important distinction from *Upeneoides bensasi* is that the habit of resting and swimming occurs in schools and not individually. Since this fish does not vibrate his tail part or drag its barbel over other fish like the catfish, it seems possible that the formation of school may be due to the visual response, as shown by PARR ('27) in several species of schooling pelagic fishes. This suspection is supported by the fact that blinded fishes show no such reaction. To clear this question, the following investigation was performed in the dark room, according to the method worked out by BOWEN ('31). The material

consisted of ten normal fishes which were brought into laboratory about a week before. A 60 watt Mazuda electric lamp which could be switched on and off at will was placed over the center of the aquarium. After a given interval of darkness, the aquarium was illuminated, and the behavior was observed as noted in the following table.

TABLE 1.

*The condition of the schooling of ten normal fishes after a given interval of darkness.*

(+ school formation; - scattered condition).

Interval	Condition	Time spent to form the school after illumination (seconds)	Interval	Condition	Time spent to form the school after illumination (seconds)
10''	+	0	2' 30''	-	6
30''	+	0	3'	+	0
50''	+	0	4'	-	8
1'	+	0	4' 30''	-	7
1' 30''	-	3	5'	-	9
2'	-	10	6'	-	10

From these experiments it seems that visual response is an essential

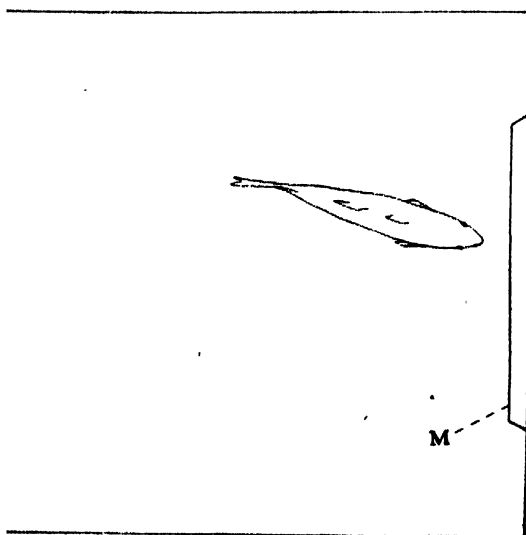


Fig. 1. Scheme showing the reaction of the fish to the mirror, M.

factor in the reaction concerned, because the schooling is broken up in about two minutes after removing of the light and then it is reformed in about 8 seconds after the aquarium is illuminated (see table).

Next, the reaction of this fish to its image on the mirror was observed to see if it shows such a characteristic reaction as found in *Morone labrax* (SPOONER, '31). A wooden aquarium, 41 cm. long, 27 cm. wide and 25 cm. deep

was prepared and the mirror, 18 by 13 cms., was erected on one of the walls. This fish became quiet in about ten minutes after being confined in the aquarium and was found to rest on the bottom with its head towards the mirror as shown in Fig. 1. Such a reaction failed when a wooden plate was replaced for the mirror. However, it must be kept in mind that six cases of negative reactions were observed in the total of twenty observations. Hence this subject needs further investigation before any definite conclusions can be drawn.

ii. *Feeding habit.* This fish is a bottom feeder and its strong reactions to the bait recall that of *Upeneoides bensasi*. This fish, however, took food at the following day after being captured in the aquarium. The two long unbranched barbels are found at the chin of this fish (Fig. 3), and the feelers are hidden under the lower jaw when the fish does not swim around searching with the barbels on the bottom.

When lugworms were dropped into the aquarium containing ten normal fishes, the fishes approached the bait by use of sight, but did not bite and swallow the lugworms readily, as do other fishes with no barbels. They swallowed the worms after touching with their feelers. Even after swallowing the food, they would continue a systematic search on the bottom with their barbels in school formation. Therefore, the reactions of the fish to pieces of lugworms hidden in the sandy bottom was tested. Again they swam along the bottom as if searching with their barbels and finally detected the hidden food in from one to five minutes. It was often noted that they would continue their active trailing with their barbels on the spot where the lugworm had laid; probably some of the body juice had escaped into the sand.

When a wisp of cotton was dropped into the aquarium, it was approached by the fishes and touched with their barbels as described above, but was never bitten by them. The fishes showed no notable reactions to a cotton saturated with 10 per cent solution of saccharose, glucose or sodium chloride. But, when the cotton was soaked thoroughly in the juice of the lugworms, it was seized by the fishes after touching with their barbels, although it was ultimately discarded and given no further notice after about five minutes, probably due to a complete diffusion of the juice into the water.

Next, two wads of white cheese cloth, closely resembling each other, were made, one containing a small stone and the other some lugworms. These wads were dropped into an aquarium with five normal fishes and observed for fifty minutes and record was kept of the number of times

each wad was bitten by the fishes. The wad with a stone was approached by them, but soon dropped. The other, while contained meat was seized and tugged by them thirty-five times in the course of the test. Another set of five normal fishes was tested in this manner and with similar results.

These results would indicate that this fish recognizes its food through some chemical sense, while its barbels seem to be important in sensing its food on the bottom.

#### EXPERIMENTS WITH THE OPERATED FISHES

To ascertain the sensibility and function of the barbel, I took from among ten normal fishes already tested five sets of two each and prepared each set differently by subjecting its members to a special operation. The following experiments were all performed in five aquariums of the same size, viz., 45 long, 30 wide and 24 cms. deep.

i. *The blinded fish.* In blinding, both eyes were destroyed by a hot needle under the anaesthetization with 5 per cent solution of ether sea water. On returning the operated fishes to the aquarium, they began in a few minutes to swim lazily about. A week after treatment of this kind, the fishes swam trailing their barbels on the bottom in normal fashion, though they occasionally collided with the walls and the schooling was not given by them. When the lugworms were dropped into the aquarium, they were detected by the fish sweeping on the bottom with its barbels after the worms fell to the bottom, although the fish was unable to pounce upon the bait falling from above.

When a fine glass or wooden rod was carefully inserted into the aquarium and brought near a blinded fish, the fish showed no reactions. Also it failed to show such a notable response to metallic rods as found in the case of catfishes (PARKER, '27; UZUKA, '34). These results seem to indicate that the barbel seems to be hardly sensitive to tactile stimuli.

For the purpose of making clear the sensibility of the barbel to chemical stimuli, the following experiments were done by the use of a fine-pointed pipette, directing a jet of various solutions tinged with methylen blue against the barbel of the fish as shown in Fig. 2, and the fish was then closely watched. Notwithstanding the most careful manipulation, a small amount of the solution could not be prevented from being swept into the mouth of the fish by respiratory current and, therefore the

gustatory organs in that cavity were stimulated.

At first the fishes were tested by distilled water, but they merely ignored or avoided it. When 10 per cent solution of saccharose, glucose and sodium chloride were applied, they showed a slight interest to it, but occasionally performed to trail on the bottom with their barbels for only short time. If, however, they were tested with a solution filtered from a mixture of freshly chopped lugworms and tap-water, they always instantly reacted as they did to meat and no amount of training would suffice to cause them to discontinue the reaction to this lugworm juice.

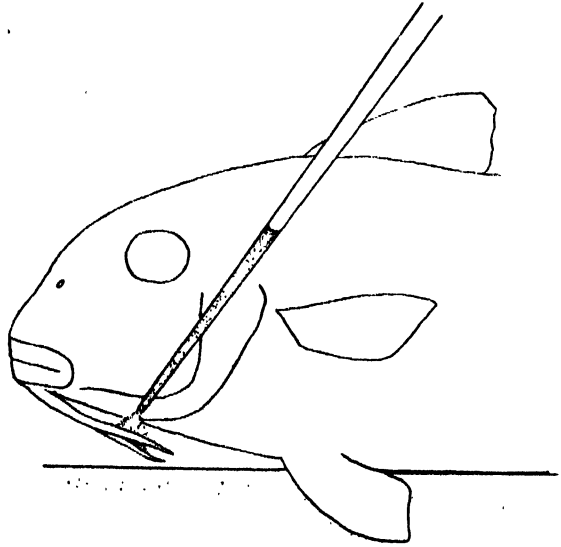


Fig. 2. Illustrating the experiment to discharge a jet of the solution against the barbels by the use of a pipette.

ii. *The fish with its barbels removed.* The two barbels were removed from the chin by sharp scissors, without anaesthetization. These fishes recovered easily and quickly, and seemed to be normal, excepting that they floated in mid-water or near the top more frequently than the normal ones did. When the worm was given, these fishes detected and pounced upon it by the sense of sight. But these fishes failed to recognize the worms hidden in sandy bottom. Moreover, they occasionally pursued and took a wisp of cotton into the mouths as eagerly as if it was meat, whereas the normal ones only touched it with their barbels and refused to take it into the mouths. The evidence from these experiments seems to favor the view that sight will not explain the final discrimination between edible and inedible material, although it plays an important part in approaching the food which falls from above.

iii. *The fish with sense of smell and sight eliminated.* To clear the sensibility of the barbel more fully, the eyes and olfactory organs of the fishes of this set were made functionless by an operation: the eyes were destroyed with a hot needle and the olfactory nerves were cut by incision

just posterior to the olfactory pits. At the expiration of a week, they were carefully inspected and tested. They swam about trailing on the bottom with their barbels and detected the lugworms as much as normal ones did. Therefore they appeared to be in satisfactory condition for experimentation.

The tests were begun by introducing a clear glass rod some 2 mm. in diameter into the aquarium and bringing it under the chin of the fish. But, no remarkable responses were given to its presence, whereupon they usually swam when the rod was actually in contact with the barbels. Similar reactions were observed when wooden and metallic rods were used in place of glass. If the electrodes with a current measuring about 5 microamperes were near to the fish, they would turn away.

I next endeavored to ascertain the effect of the chemical stimuli by the discharge of a small amount of various solutions against the barbels as shown already. On applying distilled water to these fishes, no response was observed. Even when the solutions of 10 per cent of glucose, sodium chloride and quinine were now discharged direct to the barbels, these fishes showed only slight responses to them. On being applied with a weak acid solution, they commonly turned toward it and this response was more striking when the more concentrated solution was used. These experiments were repeated several times and always with the similar results. In contrast with these applications, the solution filtered from a mixture of lugworms and the tap-water was given to these fishes. They showed a remarked degree of excitement as soon as this solution was applied, and began a systematic search over the bottom with their barbels in normal fashion. Furthermore, it is interesting to note that the fishes were able to distinguish one wad with meat from the other with nothing.

The tests here recorded show that the barbels are more sensitive to a chemical stimuli than the tactile, in other words, this organ may function as a chemoreceptor which is serviceable in sensing food.

iv. *The fish blinded and its barbels removed.* To get some clue as to whether or not the olfactory apparatus is related in sensing food as in the cases of catfish, killifish (PARKER, '10, '11), swellfish (COPELAND, '12) and smooth dogfish (SHELDON, '11), I observed the reactions of these fishes in sensing food at the end of ten days after the operation. These operated fishes were more restless and swam about more frequently than the normal fishes. If a few fragments of freshly chopped lugworms were brought near to such individuals, they showed slight excitation even before touching the food. But, they were unable to easily pounce upon

any fragments or to detect readily the lugworms on the bottom, though they often swam within a few centimeters of the food. During this period the fishes swam about excitedly in the lower part of the aquarium, in various directions, and eagerly searched for food with their mouths in contact with the bottom. They, however, were only able to take the bait into their mouths when it was near enough for their lips to come in contact with the wire to which a lugworm was hooked, or when they accidentally touched the worms with their lips. These fishes failed to recognize the food hidden in sand.

By means of pipette, a jet of distilled water was directed over the olfactory aperture of a given fish and then similarly applying a jet of lugworm juice. In the former case the jet was ignored; in the latter, the fish showed excitement and swam about in the aquarium. But, I have made no accurate observations as to whether the olfactory apparatus of this fish is serviceable in distinguishing two wads of cloth as described above, because they usually swam past the wads without noticeable change and when they came in sudden contact with the wads, as rapid avoiding reaction occurred.

It was noticed in this experiment that this fish had rather more difficulty than the fish with barbels in finding food, although the olfactory apparatus was concerned in sensing the location of food.

v. *The fish with the sense of sight and smell eliminated, and the barbels removed.* These operated fishes swam lazily and on the whole showed what might be called a nervous disposition on slight provocation. In their reactions these fishes also resemble those just described above, except that they took much more time in detecting food. This fish could take the worms only when they came in contact with its mouth. If the jet of the worm juice was discharged to these fishes, they showed less active reactions compared to normal fishes, although it might be argued, of course, that the effects of the operations upon the fishes made them unable to respond inactively.

#### HISTOLOGICAL STRUCTURE OF BARBELS

For fixation of the barbel, Bouin's solution and 10 per cent neutral formalin solution were tried and section were differentiated by the stains of widest applications. Bielschowsky's method was also applied.

The barbel of this fish is tinged with light yellow, but is not so brightly yellow as that of *Upeneoides bensasi*, and its distal end does not



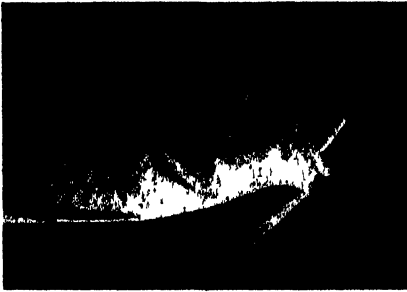


Fig. 3. Side view of the head, showing two barbels at the chin, natural size.

reach to the posterior margin of the subopercular bone (Fig. 3). The structure of this barbel, however, shows a striking resemblance to that of *Upeneoides bensasi*.

The cutaneous taste buds are buried numerous in the epidermal cells and are generally more thickly populated on the distal parts than on the proximal ones (Figs. 4 and 5). They are flask shaped, about  $110\mu$  in height and  $70\mu$  in width. The organizing elements of the taste buds divide into the sensory and supporting cells. The former is extremely attenuated above the level of its nucleus which lies near the proximal end of the cells. The sensory cells are arranged side by side situated upon the



Fig. 4. Tangential section of the distal portion of the barbel, showing the cutaneous taste buds thickly populated.  $\times 100$ .

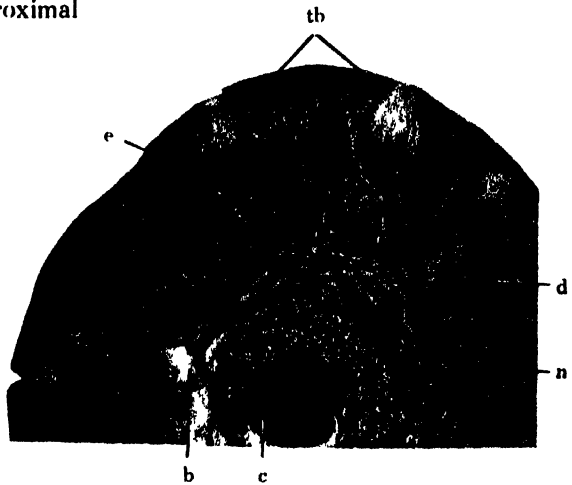


Fig. 5. Transection of the barbel, showing its structure.  $\times 150$ . *b*, blood vessel, *c*, cartilage; *d*, dermis, *e*, epidermis; *n*, bundles of nerve fibres; *th*, cutaneous taste buds.

papillar eminences of the dermis. The supporting cells cover and surround the sensory cells. Excepting the taste buds, no other notable structures are found among the epidermal cells.

At the central core of the dermis, a rod of cartilage is situated and stretches from the base of the barbel to its distal end. The large nerve trunk occupies the greater part of the dermis and encloses the cartilaginous rod. This trunk gives off numerous side branches in its course from the base of the barbel to its tip. These side branches reach the bases of the dermal papillae through the connective tissue, where the taste buds are found. This nerve trunk seems to be a branch of ramus mandibularis VII. In this point, another report will appear. Among the connective tissue, the blood vessels are also found.

According to HERRICK ('03), the cutaneous taste buds are organs of taste, and the delicacy of the sense of taste in the skin is directly proportional to the numbers of the cutaneous taste buds in the area in question. Hence, it may be also expected from the results of the histological observations that the barbel plays an important rôle in search for edible substances, for the barbel bears a great number of the taste buds on its epidermis.

#### DISCUSSION AND CONCLUSIONS

One peculiarity of this fish is that it is a schooling fish. By experimentation I have shown that blinded fishes do not form schools and in addition it was shown in Table 1 that the schooling is broken up in about two minutes after the light is removed, though a few exceptional cases were observed in the course of my tests. Furthermore, this reaction is not effected by the elimination of the olfactory sense, and this fish does not vibrate its tail in pushing against another as in the case of *Amiurus melas* (BOWEN, '31). For these reasons, I may conclude that the eyes are extremely important for schooling and that without them this reaction does not occur, as shown by PARR ('27) in the schooling pelagic fishes.

SPOONER ('27) reported already that *Morone labrix*, a schooling fish, shows a characteristic reactions to the mirror. However, in my observations on this point, all of these fishes did not react with the same regularity to the mirror.

Another feature of this fish is that it has two notable barbels at the chin, which show a striking resemblance in its structure and function to

that of *Upeneoides bensasi* (SATO, '37 a, b).

The normal fishes swim in trailing their barbels on the bottom and detect hidden food in the sand in a short time. This ability to recognize the presence of the hidden food was not hindered by the elimination of the sense of smell and sight, but the loss of this ability was caused only when the barbels were removed. In addition to this, the possibility to distinguish one wad with meat from the other without any was not influenced by making the olfactory apparatus and eyes functionless. Although, the experiments and observations here recorded show that the sight plays a major part in approaching food falling from above, and that the olfactory apparatus is serviceable for locating food substances, these two sense organs are serviceable in the preliminary steps of procuring food. Because the fish without barbels occasionally took a wisp of cotton into the mouth as eagerly as if it was meat, whereas the fish with barbels showed no such procedure. Moreover, the fishes with the barbels removed have more difficulty than those with barbels in finding food on the bottom: and the ability to discriminate between two wads was rendered decreased by removing the barbels. From these results of experiments on the barbel, this organ may be regarded as a limiting factor in the ability of this fish to recognize hidden food.

The sensibility of the barbel was also confirmed by my tests on the fishes with barbels but with the sense of smell and sight eliminated. These fishes paid no attention to the presence of a clear glass, wooden or metallic rods, and they usually swam away when these rods were actually in contact with their barbels. If the solution of saccharose, glucose, sodium chloride and quinine were discharged against the barbels of such individuals by means of a fine pointed pipette, these fishes exhibited no notable excitation. But occasionally they reacted to these solution in moving of their barbels lazily. On applying weak acetic acid solution, a rapid avoiding reactions occurred. If, however, the solution filtered from a mixture of lugworms and tap-water was used in place of these above mentioned solutions, the fishes always showed a marked degree of excitement, and began a systematic search on the bottom with their barbels. These experiments were repeated several times with the similar results. These tests show conclusively that the barbel is more sensitive to chemical than tactile stimuli. In other words, the barbel may function as a gustatory organ which responds to a chemical stimulation by substances in solution emanating from the meat on the cutaneous taste buds buried on its epidermis. Accordingly, this barbel also seems

to bear considerable resemblance of the free rays of pectoral fin of *Trigla corax* (SCHARRER, '35) in the sensibility and function.

In conclusion, then, it can be stated that the barbel is a limiting factor in sensing hidden food and this fish seems to detect his food chiefly, if not perfectly, by exploring the bottom with the feelers even in natural habitat as similar as that observed in the aquarium, judging from the fact that this fish is a bottom feeder.

#### SUMMARY

1. The barbel which is provided with the cutaneous taste buds numerous on its epidermis is more sensitive to chemical than tactile stimuli.

2. This organ seems to be absolutely necessary to recognize the hidden food and this ability is not hindered by making the olfactory organs and eyes functionless.

3. This fish shows a schooling behavior and the vision is an important factor in the formation of school, because blinded fishes give no such reaction and normal ones fail to form schools in the dark.

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# THE TEMPERATURE LIMIT OF ACTIVITY OF THE STRAWBERRY WEEVIL, *ANTHONOMUS BISIGNIFER* SCHENKLING

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(With two figures)

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## INTRODUCTION

The diurnal rhythm of the activity of the Strawberry Weevil, *Anthonomus bisignifer* SCHENKLING, was distinctly recognized in the previous investigation (KATÔ, 1937), and in which it was concluded that this diurnal activity depends mainly upon the variation of the temperature factors such as the solar radiant energy, the air temperature and the soil surface temperature. Accordingly it may be seen that the activity of this weevil is the temperature reaction (KATÔ, 1938). Now, the investigation on the temperature limit of activity of this weevil must be therefore taken into consideration in order to clarify the diurnal activity of the said weevil. In this present paper, I deal with the results of the experiments executed concerning the temperature reaction of the said weevil under the slowly rising temperature.

Before proceeding further, I wish to express my sincere thanks to Professor Dr. SANJI HÔZAWA for his cordial guidance and encouragement. I am also indebted to Assistant Professor Dr. ISAO MOTOMURA for his valuable suggestions and encouragement.

## MATERIAL AND METHODS

The overwintered Strawberry Weevil, *Anthonomus bisignifer* SCHENKLING, which were active in the strawberry garden, i. e. Gasen-En, a strawberry garden situated on Mt. Dainenji, Sendai, were used in the present investigation.

With the purpose to study the temperature limit of activity of the said weevil, four or five ones were obtained in a glass tube. That tube 2.5 cm. in diameter and about 5 cm. in length was ventilated through a

little glass tube 0.3 cm. in diameter. The air temperature in the glass tube was first lowered till 2°C and, then, allowed to rise at the rate of 1°C during every four minutes. It was, then, recognized that the troubles occurring during the experiment, i. e. the troubles concerning the balance between body temperature and the environmental air temperature or between the environmental air temperature and the reading of the thermometer, may be taken away by this slow rising of the environmental temperature.

Then a similar experiment was conducted with the purpose of measuring the locomotion velocity of the weevil in various temperatures. In this case a glass tube of about 1.5 cm. in diameter and about 15 cm. in length, in which the weevils were allowed to climb up from the bottom to the top, was used. Accordingly, as an index of the locomotion velocity, climbing velocity was dealt with in this present investigation.

## RESULTS AND DISCUSSION

### *1) The Temperature Limit of Activity*

The experiments concerning the temperature limit of activity of various insects have been done by many investigators (BERTRAM, 1935; BODENHEIMER & KLEIN, 1930; BRAES, 1927; CHAPMAN and others, 1926; HÔZAWA, 1929; MISHIMA, 1936; MOTOMURA, 1936; PARKER, 1930; etc.). According to the results obtained by the said investigators, it is recognized that the insects living in different environments, have to each other characteristic minimum and maximum temperature limits of activity.

When the temperature is gradually lowering, the activity of the Strawberry Weevil becomes weaker and weaker till at last it becomes motionless and unable to stand. The air temperature in the glass tube was, then, slowly raised at the rate of 1°C in every four minutes, as already mentioned. When the temperature is allowed to rise, we remark first that the weevil performs a slight movement of the antenna or the leg, then, it stands up on its feet, and, after a while, begins to crawl. When the temperature continues to rise, the activity of the weevil rises until it becomes excited and nervous by heat and finally unable to stand.

In the present investigation the various activities of the weevil were recorded to indicate the five following conditions:

**A:** The observation of the first slight movement of the leg or the antenna of each individual.

- B:** The standing of each one on its feet for the first time.  
**C:** The beginning of the crawling of each individual.  
**D:** The flying of the first individual.  
**E:** The falling down of each weevil becoming unable to stand by heat paralysis.

In the case of measuring the temperature, indicating the condition of a certain activity, the temperature, at which the first or the last individual indicates the condition of the said activity, has been ever observed. Since this method seems, however, not so much biologically important, the average value of the temperature, at which each individual indicates the said condition of the activity, was, in the present experiment, taken up as the one showing the said condition of activity. As this method was not available for the case of the beginning of the flying, the temperature, at which the first individual flew, was used as ever.

**A-condition** of this study corresponds to CHAPMAN's "Point I" and to BODENHEIMER's "II-condition"; **B-condition** to the former's "Point II"; **C-condition** to the former's "Point III" and to the latter's "III-condition"; and **E-condition** fits for CHAPMAN's "Point VII" (Table 1) (CHAPMAN and others, 1926; BODENHEIMER, 1930).

TABLE 1  
*Comparison of the conditions of activity used by various investigators*

remarks	Condition of activity		
	KATÔ's	BODENHEIMER's	CHAPMAN's
The first slight movement of the leg or the antenna	A	II	I
The standing of each one on its feet	B	—	II
The beginning of the crawling	C	III	III
The beginning of the flying	D	—	—
The falling down by heat paralysis	E	VII	VII

(i) The results obtained in the present investigation concerning the Strawberry Weevil are tabulated in Table 2 and shown in Fig. 1.

According to these results, **A-condition** of this weevil is 4.67°C, which is nearly similar to 4.5°C shown in the case of the Rice Weevil, *Calandra oryzae* (L.) (HÔZAWA, 1929), but is distinctly low comparing with 7.8°C in



TABLE 2

*Frequency distribution showing the various conditions of activity of the Strawberry Weevil*

Temperature	Frequency (%)			
	A-condition	B-condition	C-condition	E-condition
2.4°C	3.6			
3.2	0.0			
4.0	57.1			
4.8	35.7			
5.6	3.6	3.6		
6.4		25.0		
7.2		17.9	14.3	
8.0		17.9	7.1	
8.8		28.6	21.4	
9.6		7.1	21.4	
10.4			25.0	
11.2			7.0	
12.0			3.6	
*				
*				
*				
*				
39.0				
40.0				6.7
41.0				13.3
42.0				33.3
43.0				26.7
44.0				13.3
45.0				6.7
46.0				

the case of the Desert Locust, *Schistocerca gregaria* FORESK (BODENHEIMER, 1930) and also with 7.0°C of the American Grasshopper, *Melanoplus mexicanus mexicanus* SAUSSURE (PARKER 1930).

(ii) The temperature indicating the B-condition at which the weevil stands up on its feet is 8.10°C. As clearly shown in Table 2 and Fig. 1, it is noticeable that two maximum values are recognized in the frequency distribution. This may be easily understood from the fact that some weevils, used in the experiment, were able to stand up at the first trial, but the others could only do it after several repeated trials. That is to say, the greater part of the weevil which stood up at the first trial belong to the first frequency distribution and the others to the second distribution. The exact temperature at which the weevil is able to stand may be therefore lower a little than 8.10°C. Comparing this result with that shown by other investigators, it is clear that this is fairly higher than that shown by the Greenland insects which are set in full activity

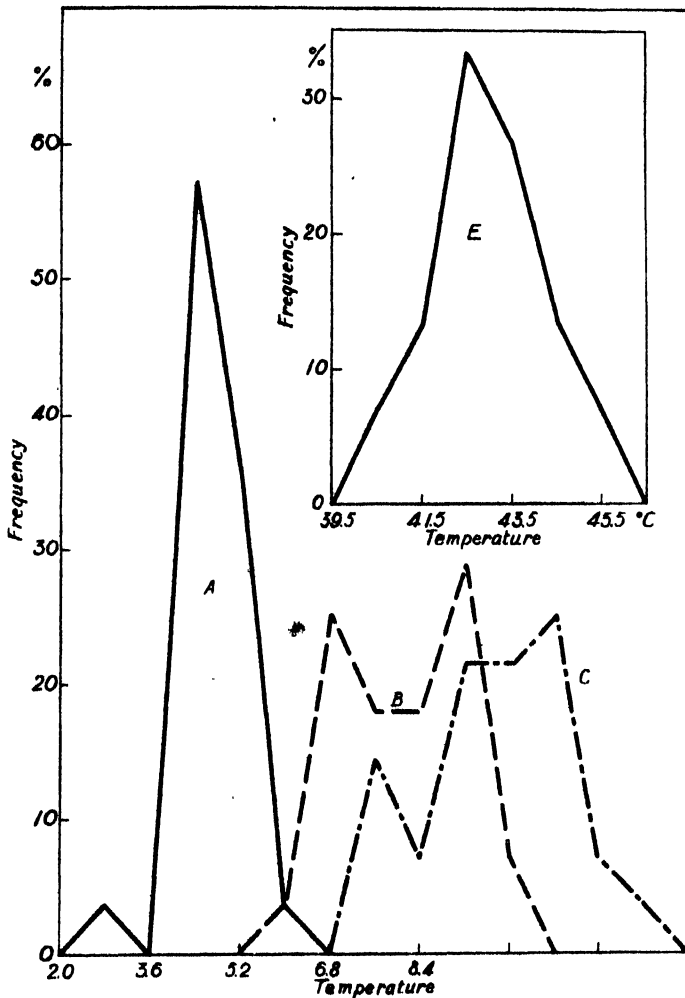


Fig. 1. Frequency polygons showing the various conditions of activity of the Strawberry Weevil. A: A-condition; B: B-condition; C: C-condition; E: E-condition.

at 10°C (BERTRAM, 1935), but is distinctly lower than 10°C shown by the American Grasshopper (PARKER, 1930).

(iii) C-condition at which the weevil begins to crawl is observed at 9.70°C. This is lower than that shown by the Desert Locust and again than that of the American Grasshopper. The beginning of the crawling was observed at 10°C in the former case and at 12°C in the latter. In

the case of the Strawberry Weevil, the influence of the phenomenon, which was observed in the preceding *B*-condition, is seen; i. e. weevils, which belongs to the first of the frequency distribution obtained in the *B*-condition, begin to crawl earlier than the others.

(iv) When the temperature rises continuously, the crawling of the weevil becomes gradually active till 22.8°C, at which degree the beginning of the flying of the first individual is observed. The temperature, at which the beginning of the flying is seen, agrees fairly well with that of *Rhaphidopalpa femolaris* MOTS., i. e. about 22°C (MISHIMA, 1936).

(v) The temperature of *E*-condition, at which the weevil becomes unable to stand by paralysis, is 42.9°C. But in the case of the Desert Locust (BODENHEIMER, 1930), it is 49.6–50.9°C and in the case of the American Grasshopper (PARKER, 1930), 47°C. Only in the case of *Rhaphidopalpa* (MISHIMA, 1936) it was 41–44°C which may rather be similar to that of the Strawberry Weevil. In the case of the Hotspring Insects, it is expected that the maximum temperature limit of activity may be remarkably higher comparing with others. According to BRAES, the maximum temperature limit of *Chironomus* sp. was actually very high showing 49–50°C (BRAES, 1927), and in *Stratiomya japonica* VAN DER WULP it was 50.8°C (MOTOMURA, 1936). These are distinctly higher than that of the Strawberry Weevil.

(vi) Summarizing the above mentioned results, in the case of the Strawberry Weevil, *A*-condition is 4.67°C, *B*-condition 8.10°C, *C*-condition 9.70°C, *D*-condition 22.8°C, and *E*-condition 42.92°C. According to these results, it is clearly recognized that the minimum and maximum temperature limits of activity in the case of the Strawberry Weevil, are both remarkably low comparing with those of the Desert Locust or the American Grasshopper which seems to be rather tropical insects. Similar relation is observed with the Hotspring Insects. On the contrary, minimum temperature limit of activity of the Strawberry Weevil is higher than that of the Greenland insects, the arctic insects. But the similar degree of the temperature limit of activity was observed only in the cases of the Rice Weevil and *Rhaphidopalpa* (*Chrysomelid*).

## II) The Temperature Zone of Activity

The temperature zone of activity of the Strawberry Weevil may be easily measured from the difference between *C*-condition and *E*-condition,  
i. e. .... 42.92° – 9.70° = 33°C.

According to "Study in the Ecology of Sand Dune Insect", which was written by CHAPMAN and the others, each insect, which comprises the sand dune fauna, is not similar when compared on the basis of the temperature limit of activity (CHAPMAN and others, 1926; CHAPMAN, 1931). According to this, the climate of the sand dune is essentially like that of the desert. It may therefore distinctly be seen that the maximum and minimum temperature limits of the Strawberry Weevil are low comparing with those of the various insects of the sand dune. Only in the cases of the nocturnal insect, *Geopinus incrassatus* DEJEAN, and of *Melanoplus femur-rubrum* DEG. the minimum temperature limit of activity is similar to that of the Strawberry Weevil; but, the temperature zone of activity of both species is also wider than that of the latter, showing 39°C and 43°C respectively. This narrow temperature zone of activity in the case of the Strawberry Weevil shows that the adjustment to high temperature is weak comparing with the above mentioned sand dune insects.

### III) Locomotion Velocity of the Strawberry Weevil

In the range of the temperature zone of normal activity, the degree of the activity is not similar under various temperature conditions, but it becomes more active with the rising of the temperature. This fact is also distinctly known by what was above mentioned. I measured, however, the velocity of the locomotion in climbing under the gradually rising temperature, with the purpose to investigate quantitatively the degree of the activity.

The relation between the temperature and the locomotion velocity of various insects was examined by many authors (BĚLEHRÁDEK, 1935; BODENHEIMER & KLEIN, 1930; CROZIER, 1924; MILLER, 1929; PRZIBRUM, 1909; SHAPLEY, 1920; STUMPER, 1922). These results pretty well agree with the formula derived from VANT HOFF's Law and ARRHENIUS' Law. According to BODENHEIMER & KLEIN, in the case of the "Ernte Ameise", *Messor semirufus* E. ANDRÉ the locomotion velocity curve represents the hyperbola and the "aktivität Nullpunkt" was never constant through the year, but varies, showing different values in each month (BODENHEIMER & KLEIN, 1930).

The results obtained in the present investigation, were shown in Fig. 2 and Table 3.

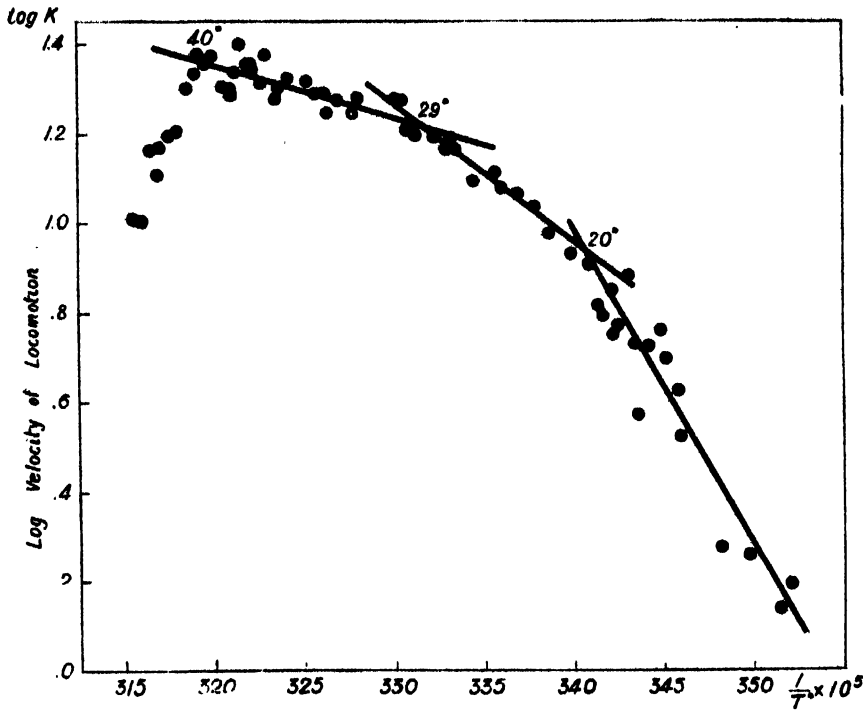


Fig. 2. Locomotion velocity of the Strawberry Weevil.

TABLE 3  
*Locomotion velocity of the Strawberry Weevil*

Temperature	Velocity (cm./sec.)	Temperature	Velocity (cm./sec.)
11.0°C	.1563	29.0°C	1.5790
13.0	.1818	30.0	1.9048
14.2	.1901	34.0	1.9444
16.0	.3333	36.0	2.0000
17.6	.5357	38.0	2.5000
19.0	.5932	38.7	2.0000
20.0	.6593	40.0	2.2727
23.0	1.0909	40.5	2.1622
25.0	1.3043	42.0	1.5686
27.5	1.5152	43.0	1.4607

The curve of the locomotion velocity under various temperatures may be represented by the **ARRHENIUS'** formula :

$$K_2 = K_1 e^{\frac{\mu}{2}(T_1 - T_2)}$$

Here,  $K_1$  and  $K_2$  represent the velocity in the case of the absolute

temperature  $T_1$  and  $T_2$  respectively.

Thus, the critical temperatures were calculated. These were 20°C and 29°C, and the thermal increment was as follows:

Below 20°C..... $\mu=36060$

20°C—29°C..... $\mu=13800$

Above 29°C..... $\mu=5400$

It may be interesting to see that the first critical temperature is similar to the temperature at which the weevil begins to fly, showing 20°C and 22.8°C respectively.

### SUMMARY

1. In the present paper, the investigation concerning the temperature limit of activity of the Strawberry Weevil, *Anthonomus bisignifer* SCHENKLING, was done with the purpose to clarify the diurnal activity of the said weevil.

2. The experiment was conducted under the gradually rising temperature at the rate of 1°C in every four minutes, using the overwintered weevils which were active in the strawberry garden.

3. The temperature, at which the slight movement of the antenna or the leg is observed, is 4.67°C, the one at which the weevil stands up on its feet is 8.10°C, and the one at which the beginning of the crawling is observed is 9.70°C. The beginning of the flying is seen at 22.8°C. The temperature at which the weevil falls over by paralysis is 42.92°C. Accordingly the temperature zone of activity is about 33°C.

Comparing the Strawberry Weevil with the Desert Locust or with the American Grasshopper, the maximum and minimum temperature limits of the said weevil are remarkably low, but higher than those of the Arctic Insects of Greenland. It is suggested that this temperature reaction is generally similar to those expressed by the Rice Weevil and *Rhaphidopalpa*.

4. The locomotion velocity, measured under various temperatures, agrees with the ARRHENIUS' formula. The critical temperatures are at 20°C and 29°C, and the critical thermal increments ( $\mu$ ) are as follows:

Below 20°C..... $\mu=36060$

20°C—29°C..... $\mu=13800$

Above 29°C..... $\mu=5400$

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# THE DIURNAL ACTIVITY OF THE STRAWBERRY WEEVIL, *ANTHONOMUS BISIGNIFER* SCHENKLING, WITH A NOTE ON THE ECOLOGICAL MEANING OF THE SOLAR RADIANT ENERGY

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## INTRODUCTION

It is evident that the diurnal rhythm is seen in the activity of the Strawberry Weevil, *Anthonomus bisignifer* SCHENKLING, especially in the egg-laying activity which is dealt with as an index of the general activity. That is to say, according to the statistical investigation, from total number of eggs laid every day, 88.4 per cent are deposited in the daytime, and only 11.6 per cent are laid in the night (Table 1).

TABLE 1

*The diurnal activity of the egg-laying of  
the Strawberry Weevil*

Time-interval	Number of eggs (%)
h h 6.00-10.00	22.6±1.2
10.00-14.00	37.1±1.7
14.00-18.00	28.7±1.8
18.00- 6.00	11.6

Besides, concerning the activity in the daytime, 22.6 per cent of the daily total number are oviposited in the morning (6 am.-10 am.), 37.1 per cent about noon (10 am.-2 pm.), and 28.7 per cent in the afternoon (2 pm.-6 pm.). We may therefore easily conclude that the Strawberry Weevil is a diurnal insect.

It is almost doubtless that the diurnal rhythm of the insect activity depends, in many cases, upon the diurnal variation of the climatic factors. But the problem is to find out which of these climatic factors is most effective upon the insect activity.



The investigation concerning the correlation between the egg-laying activity of the said weevil and the various climatic factors was carried on, since a few years by myself. In this investigation the main effective climatic factors were discussed statistically, calculating the coefficient of correlation (KATÔ, 1936 & 1937 a). The evaporation, humidity, air temperature, soil surface temperature, and the solar radiant energy were microclimatically measured, and then the correlation between the egg-laying activity and these measurements was investigated. The conclusion was that the temperature factors, such as the solar radiant energy, the air temperature and the soil surface temperature, are the most important. Conclusively, it was suggested that the said activity might mainly go with the temperature reaction. This fact was also distinctly recognized in the investigation carried in the case of the solar eclipse, using the said weevil (KATÔ, 1937 b).

It may be consequently permissible to conclude from what was above mentioned, that the solar radiant energy which has been almost neglected in the field of the ecology must be recognized as one of the temperature factors of the environmental factors.

According to the experiment made on the temperature limit of the activity of the said weevil (KATÔ, 1938), the minimum effective temperature is 9.70°C and that activity becomes more strong with the rising temperature and then at 22.8°C the beginning of the flying is observed.

In the present investigation I deal with the observation concerning the diurnal activity of the Strawberry Weevil. Taking the relation between the activity and the temperature factors into consideration, a special study refers to the process of transformation which goes from the nocturnal resting condition to the diurnal active one.

I wish, here, to express my sincere thanks to Professor Dr. SANJI HÔZAWA for his kind instructions which I received in the course of this investigation, and to Assistant Professor Dr. ISAO MOTOMURA for his valuable criticisms. I am also indebted to Mr. T. MIYAZAWA, the owner of the strawberry garden, for his kind consideration.

#### METHODS AND MATERIAL

The investigation was made in 1936 and 1937 at Gasen-En, a strawberry garden situated on Mt. Dainenji, Sendai, (Fig. 1) during the season of activity of the Strawberry Weevil, *Anthonomus bisignifer* SCHENKLING.

In the field experiments, a glass frame (100 × 60 × 50 cm.) was used

for the convenience of the observation. For the ventilation purpose, a wire net (0.5 mm.) was stretched on the four sides of the base belt of

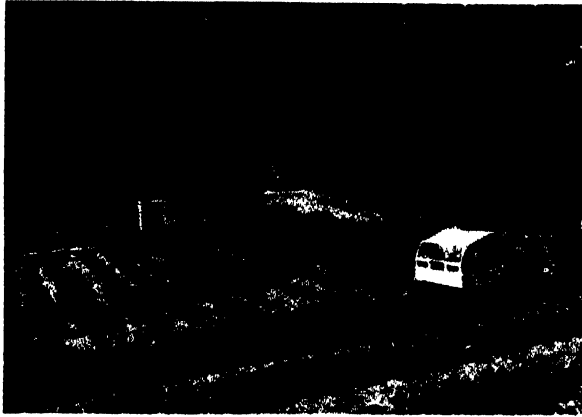


Fig. 1. General view of the strawberry garden showing the experimental frame in the right hand.

the frame and also at the upper part of that frame. Two strawberry plants were planted inside of this frame and 10 or more weevils were put into the same. Thus the behavior and the activity of these weevils were observed. At the same time, the air temperature was measured in this frame at the height of 20 cm. above the ground, and the soil surface temperature was also observed. Then the solar radiant energy was measured by the congregated solar radiation thermometer. By using this frame, the rapid descent of the air temperature, and, consequently, the various mechanical influences caused by the evaporation or by the water drops or by any other causes (KATÓ, 1937 a) were possibly removed. Therefore it may be conceivable that, in the present experiment, the temperature factors may have a main effect upon the activity of the said weevil.

The locomotion velocity of this weevil was also measured in the frame with the purpose of comparing it with the result obtained by the laboratory experiment (KATÓ, 1938). A glass tube of 1.5 cm. in diameter and about 13 cm. in length and the several weevils coming from the laboratory work were used in this investigation. The climbing velocity was also measured as an index of this locomotion velocity.

## RESULTS

(I) In general, the Strawberry Weevil, inactive when the night falls

and motionless during the night, becomes active with the sunrise. In order to investigate the progression of the activity of the said weevil transforming from the nocturnal resting condition to the diurnal active condition, observations were carried on beginning from daybreak and continuing until the diurnal active condition was distinctly established. These observations were made eight times; i. e. at 5th, 7th, 11th, 14th, 18th, 20th, 22nd, and 23rd of May. Some records obtained by these experiments were tabulated in Table 2.

TABLE 2  
*Records of the observation of the diurnal activity  
of the Strawberry Weevil*

No. 1

7th of May, 1937.

Time	Sol. Rad. Thermom.		Air- Temp	Soil- Temp.	Sunshine	Observation
	Black bulb °C	White bulb °C				
5.15 am.	6.8	5.8	5.2	5.0	bright	No active weevil is seen. One is resting between a petiole and a stem.
5.30	9.6	7.5	7.2	5.0	..	One resting at the root of the plant moves the antenna and then begins to crawl slowly.
5.40	10.5	8.5	8.4	5.2	..	The said weevil migrates slowly into the rays of the sun.
6.10	18.7	13.6	11.5	6.5	..	One weevil resting on the injured flower-bud which had fallen on the ground moves the antennae. General activity is very weak.
6.15	19.8	14.5	13.2	7.0	..	One weevil crawls up from a leaf-sheath and after a while crawls again into that leaf-sheath. Two weevils crawl up from the part of the root, migrate into the rays of the sun and do cleaning movement. The weevils resting in the sunshine begin to crawl and become active earlier than the others resting in the shade.
6.25	21.2	15.4	14.8	7.2	..	Two weevils crawl or move the antennae in the sunshine at the lower part of the stems.
6.30	22.2	16.0	15.0	7.9	..	One pair of mating and one more weevil are in the sunshine and are somewhat active. One resting in the shade does the cleaning movement slowly.
6.40	23.8	17.9	17.0	9.3	..	Two weevils crawl up to the flower-buds, two pairs of mating are on the petiole. One resting in the leaf-sheath begins to migrate.

Time	Sol. Rad. Thermom.		Air-Temp.	Soil-Temp.	Sunshine	Observation
	Black bulb	White bulb				
	°C	°C	°C	°C		
6.45 am.	24.3	18.1	17.0	9.7	bright	10 weevils are active on plants. Weevils in the sunshine are generally in the normal activity.
7.00	24.6	18.7	17.6	11.5	..	12 weevils are in the sight. The weevils in the sunshine are in the normal crawling, but the others in the shade are in the slow crawling.
7.10	25.4	19.5	17.8	11.8	..	13 weevils are active in the sight. A few weevils are feeding on the plant.
7.30	25.0	19.3	17.4	12.5	..	Three weevils are laying eggs.
7.40	25.9	19.6	18.0	14.0	..	All weevils are generally in the normal activity.
8.00	25.6	20.0	18.6	16.2	..	The same.
8.10	26.7	20.8	19.0	16.5	..	One weevil begins to fly for the first time.
8.17	27.7	21.4	19.4	17.3	..	One more weevil flies short distance. One weevil flies from the plant to the glass wall of the frame and crawls.
8.40	29.0	23.0	21.5	19.5	..	One more flies and crawls on the glass wall of the frame. One weevil cuts down the flower-bud which was oviposited.
8.47	29.8	23.6	21.8	20.0	..	One more weevil is flying about the plant.
9.00	31.3	25.0	22.5	20.4	..	Three weevils are crawling on the glass wall of the frame. All weevils in the sight are in active condition.
9.30	31.3	25.0	23.0	21.5	..	All weevils are in full activity.

## No. 2

18th of May, 1937.

4.54 am.	10.3	10.3	10.3	10.5	cloudy	One weevil is feeding on a fruit. The other one is crawling on a leaf.
5.00	10.3	10.4	10.3	10.5	..	One weevil crawls slowly and rests on the glass of the frame. One is feeding on a fruit. Two weevils are crawling on a stem and on a leaf.
5.07	10.6	10.4	10.4	10.5	..	One crawls up a stem, one is feeding on the injured flower-bud fallen on the ground and one is resting at the lower part of the plant. One crawls slowly and rest on the glass wall of the frame.
5.15	11.0	10.7	10.7	10.5	..	One weevil crawls up a stem. The said weevil resting at the lower part of the plant begins to crawl slowly.
5.30	11.4	11.2	11.0	10.7	..	Four weevils rest and crawl at the lower part of stems or petiole. Two weevils are feeding on a fruit and a flower-bud. One is resting on the glass of the frame.

Time	Sol. Rad. Thermom.		Air-Temp.	Soil-Temp.	Sunshine	Observation
	Black bulb	White bulb				
	°C	°C	°C	°C		
5.45 am.	12.0	11.6	11.4	10.9	cloudy	All weevils in the sight are, in general, in weak activity.
6.15	12.3	11.8	11.7	11.1	"	Six weevils are on the plant crawling slowly. One is feeding on a fruit.
6.30	13.2	12.4	12.0	11.5	"	The greater part of the weevils in sight are generally in normal crawling in the middle part of the plant.
6.40	14.7	13.7	12.9	11.9	"	Two weevils crawl up from the lower part of the plant.
6.50	16.8	14.9	13.8	12.6	slightly over-casted	One pair of mating weevils are on the fruit. Two weevils are on the glass of the frame.
7.00	15.7	14.2	13.8	12.8	cloudy	There are nine weevils on the plant and half of them are crawling and others are resting.
7.15	14.4	13.7	13.2	12.7	"	All weevils are generally in normal crawling at the lower part of the plant.
7.30	14.4	13.6	13.3	12.6	"	
7.45	14.6	13.8	13.4	12.7	"	No active crawling is seen.
8.15	16.0	14.8	14.0	13.2	"	The same.
8.40	18.0	16.3	15.0	14.5	"	All weevils are in general in active condition.

## No. 3

20th of May, 1937.

5.00 am.	10.4	10.3	9.8	11.0	cloudy	One weevil is laying an egg on a flower-bud. 10 weevils on the glass of the frame are in resting condition.
5.10	10.7	10.5	10.2	11.0	"	One weevil crawls up on a leaf. Two are feeding on a flower-bud.
5.15	10.8	10.7	10.4	11.0	"	Two weevils resting on the glass wall of the frame begin to crawl. One weevil crawled up from the lower part of a stem begins to feed on a fruit. One more crawls up a stem to a flower-bud.
5.20	11.2	10.9	10.7	11.1	"	Three weevils are crawling about on leaves. The others are resting.
5.30	11.7	11.4	11.1	11.2	"	11 weevils are on the glass wall of the frame, two of them are crawling and others are resting. Two crawl about on the plant. One is feeding on a fruit.
5.40	11.9	11.4	11.3	11.5	"	One injured flower-bud falls on the ground.
6.10	12.2	11.7	11.5	11.9	"	
6.30	12.0	11.5	11.5	11.7	"	10 weevils are on the glass of the frame under resting condition. Two are on flower-bud piercing them. One crawls and two rest on plant.

No. 4

22nd of May, 1937.

Time	Sol. Rad. Thermom.		Air Temp.	Soil Temp.	Sunshine	Observation
	Black bulb	White bulb				
	°C	°C	°C	°C		
4.55 am.	8.4	8.4	8.2	8.8	cloudy	No one crawls.
5.00	8.5	8.3	8.2	8.8	"	One weevil resting at the root of the plant begins to crawl and rests again.
5.30	8.6	8.6	8.6	8.9	"	No one crawls.
6.00	8.7	8.7	8.7	9.0	"	The same.
6.30	9.5	9.4	8.9	9.2	"	The same.
6.40	9.4	9.6	9.0	9.4	"	One weevil begins to crawl between stems at the lower part of the plant and rests again in a leaf-sheath.
7.00	10.7	10.5	9.8	10.0	"	No one crawls.
7.30	11.4	10.9	9.8	10.4	"	The same.
7.43	12.0	11.2	10.0	10.5	"	One crawls up a stem.
7.50	12.8	11.5	10.2	10.6	"	Two weevils crawl about on the plant. One is piercing a fallen bud. One crawls up a stem.
7.55	13.0	11.7	10.6	10.7	"	One weevil crawls up on a leaf from the lower side of the same.
8.00	13.4	11.9	10.8	10.8	"	Five weevils are on the plant in slow crawling. Two are feeding on a flower-bud and on a fruit. Four are resting on the glass wall of the frame. One resting between stems.
8.15	13.4	12.0	11.3	11.1	"	
8.30	14.1	12.6	11.6	11.4	"	
9.00	13.4	12.2	11.7	11.5	"	One weevil begins to crawl on the glass of the frame. One crawls on plant. One is feeding on a fruit, and others are resting.
9.30	18.7	15.8	14.0	12.9	soft-sunbeam	Five weevils crawl actively on the plant. Five crawl on the glass wall. Two are feeding on fruits.
9.35	21.3	17.5	15.6	13.9	"	11 are crawling on the plant. All are generally under active condition. One pair of mating weevils and the one, which is feeding on a injured flower-bud fallen on the ground, are seen.
9.45	24.5	20.3	17.8	16.1	bright	Five or six weevils crawl up to the upper part of the plant very actively. One weevil flies short distance for the first time. One is resting in the shade.
9.55	26.3	21.7	18.8	17.4	"	One more weevil tries to fly opening the elythra. Three weevils on leaves intend to fly in the sunshine, but no flying is seen.

Time	Sol. Rad. Thermom.		Air-Temp.	Soil-Temp.	Sunshine	Observation
	Black bulb °C	White bulb °C				
10.00 am.	28.8	23.5	20.5	19.7	bright	One weevil is flying from a leaf to the glass wall of the frame. Eight are active on the glass. All weevils in sight are generally in full active condition.
10.20	34.3	27.7	23.6	24.7	"	Active crawling, flying and feeding are observed.
10.45	37.0	28.5	23.9	24.5	"	The flying is very active.
10.50	40.0	31.0	25.4	25.6	"	One weevil come from the field and attaches to the outer side of the glass wall of the frame. It seems that the flying is also very active in the field.
11.00	41.2	32.5	27.3	28.3	"	All weevils are in full activity.
11.30	41.7	33.2	28.6	31.7	"	Very active flying is seen.
12.00	40.5	32.7	27.4	32.6	"	
1.00 pm.	39.5	31.8	25.8	29.0	"	
2.00	41.5	33.4	26.7	26.7	"	
3.00	37.8	30.5	25.1	25.9	"	
4.00	28.4	25.5	24.0	24.3	"	All weevils crawling on the glass and on the plant are in active condition.
5.00	21.5	20.8	20.6	21.9	shade	12 weevils are crawling on the glass wall. Four weevils are on the plant in active crawling, but no flying is seen.
5.15	18.0	17.3	17.5	21.3	"	The activity is gradually weakening. Nine are on the plant crawling or feeding. Nine are on the glass wall. No flying is seen.

## No. 5

23rd of May, 1937.

4.50 am.	6.7	6.6	6.3	7.7	soft-sunbeam	Only one weevil crawls very slowly between stems at the lower part of the plant. Others are still in the resting condition.
4.55	7.2	6.9	6.5	7.7	"	Dormant one stands up on its feet. One resting between the stem and a petiole begins to crawl down on the ground.
5.10	8.3	7.8	7.4	8.0	"	The same weevil begins to feed on a flower-bud.
5.15	8.5	7.9	7.5	8.0	"	One begins to crawl on the injured bud fallen on the ground. One is feeding on a fruit.
5.20	8.7	8.0	7.5	8.1	"	The weevil resting in the corner of the glass wall of the frame begins to crawl in the sunshine and then stops.

Time	Sol. Rad. Thermom.		Air Temp.	Soil Temp.	Sunshine	Observation
	Black bulb	White bulb				
	°C	°C	°C	°C		
5.35 am.	10.8	8.9	8.4	8.6	soft-sunbeam	More one weevil is crawling about between stems. Two are feeding on a fruit and on a flower-bud
5.45	11.0	9.7	9.0	9.0	"	One resting in the corner of the glass begins to crawl slowly in the sunshine.
6.15	11.9	10.5	9.8	9.8	"	One weevil crawls up from the lower part of a stem.
6.20	12.3	10.7	10.0	9.9	"	More one weevil begins to crawl between stems.
6.25	13.2	11.4	10.4	10.0	"	Two crawl on the glass.
6.30	13.9	11.8	11.0	10.4	"	One crawls up on a leaf.
6.35	15.5	13.3	12.1	10.7	"	A few weevils migrate from the lower part of the plant to the middle of the same.
6.40	16.7	14.2	13.2	11.0	"	All weevils in sight are generally in slow crawling.
6.45	18.4	15.4	14.5	11.7	"	Seven are on the plant, one of them is fairly active. Three are on the glass. One is feeding on a injured bud fallen on the ground
7.20	17.7	15.4	14.7	13.0	"	Weevils in the sunshine are generally in normal activity and one in the shade is not active.
7.24	16.7	14.6	14.0	13.0	"	No crawling is seen. Three weevils are feeding.
7.38	23.3	18.9	17.3	14.5	bright	One weevil crawling on the glass wall flies to the ground. The difference of the activity is distinctly seen between the weevils in the sunshine and those in the shade. 11 weevils are on the plant, four of them are feeding. Five are on the glass.
7.45	25.5	20.3	18.0	15.0	"	All weevils are under active condition.
7.55	27.7	22.0	19.7	16.0	"	One weevil flies.
8.10	25.8	21.1	19.6	17.5	cloudy	One weevil tries to fly.
8.18	22.4	18.8	18.5	17.5	"	The said weevil flies from the leaf to the glass wall and crawls actively. One on a stem tries to fly.
8.22	25.7	21.3	19.6	17.8	bright	One weevil tries to fly opening the elytra.
8.35	29.2	23.6	21.5	20.0	"	All weevil are under full active condition.

(II) A few weevils which had been already in the active condition were observed 4.50 am. on the 18th of May and at 5.00 am. on 20th of the same month. In both days soil surface temperature, the air temperature and the reading of the black bulb of the solar radiation thermometer were at that time all above 10°C. Because it was cloudy in both days,



the solar radiant energy was very slight, though it was already after the sunrise, and the reading of the said black bulb was almost equal to the air temperature. It is, however, conceivable that the climatic condition of these both days was at about 5 am. in the suitable condition for the crawling of the weevil.

(III) On the contrary it was cooler on the 7th, 22nd and 23rd of May than on both days above mentioned; and, consequently, we could not observe any active weevils at all of 5.15 am., 4.55 am. and 4.50 am. The first slightly crawling weevil was observed at the time when the reading of the black bulb was 7.2°C, the soil surface temperature being 5.0°C and the air temperature showing 6.5°C. The average was 8.4°C, 7.2°C and 7.3°C respectively.

(IV) It seems very noticeable that the distinct difference in the process of the transformation from the resting condition to the active one was observed between the fine day and the cloudy day. In cloudy weather the said transforming process gradually and uniformly progresses all over the plant, while on the contrary, in fine weather, the progression is different in various places of the plant. In consequence, it is evidently shown that the weevil resting in the sunshine begins to move earlier than the one resting in the shade; consequently it is observed very often that on one hand the weevils are already in full active at the place where the sun shines, but on the other hand the weevils which are in the shade do not move or show only very slight movement.

(V) It is also noticeable that the crawling weevils often migrate into the place where the sun shines directly.

(VI) When the air temperature rises above 15°C and the reading of the black bulb is above 20°C, the general activity of the Strawberry Weevil becomes active. Then the beginning of the flying was observed at about 19°C of the air temperature and at about 25°C of the black bulb, the average being 18.0°C and 24.8°C respectively. On the 7th of May the beginning of the flying of the weevil was observed at 8.40 am. and on the 22nd at 8.45 am. On the 18th of May the flying was not yet seen at 8.40 am., but on the 23rd it was already observed at 7.38 am., the weather being very fine. The first weevil beginning to fly, in the fine day, was always seen in the rays of the sun.

(VII) As is distinctly shown in Table 2, the feeding, the mating and the egg-laying begin in parallel with the beginning of the general activity; accordingly the egg-laying activity seems to be recognized as an index of the general activity.

(VIII) Thus in the daytime the crawling, the flying, the feeding, the mating and the egg-laying are very actively done. When the weather is cool and cloudy it is easily conceivable that the general activity is very weak and consequently no flying is done through one day. On the rainy day the activity is almost inhibited as already seen in previous paper (KATÔ, 1937 a).

Even in the fine weather the activity decreases gradually in the afternoon, but in this case the decreasing progression of the activity is very slow comparing with the increasing progression observed in the course passing from the night to morning.

(IX) Some of the results obtained from the investigation measuring the locomotion velocity were tabulated in Table 3 and shown in Fig. 2.

TABLE 3  
*Locomotion velocity of the Strawberry Weevil measured under the field condition*

Time	Velocity cm./sec.	Solar Radia. Thermom.		Air-temp.	Soil-temp.	Sunshining
		Black bulb	White bulb			
5.25 am.	.210	14.5°C	13.0°C	13.0°C	12.3°C	bright
5.36	.550	19.8	15.3	14.8	13.0	"
5.43	.365	16.0	14.3	14.6	13.1	cloudy
6.35	1.087	26.8	19.5	20.0	16.7	bright
7.25	.986	25.0	21.8	21.7	23.5	cloudy
7.51	1.429	29.5	24.0	23.4	24.5	bright
8.00	1.667	31.4	25.7	25.3	25.3	"
8.49	1.250	30.0	25.9	27.1	28.2	cloudy
9.18	2.000	34.3	27.9	27.2	29.5	bright
9.40	1.842	33.2	27.5	27.0	30.9	"

The locomotion velocity was represented by the climbing velocity as in previous paper (KATÔ, 1938). In fine weather the weevil becomes suddenly active after daybreak and therefore the velocity of climbing increases rather rapidly. It is very often observed and noticeable that the climbing velocity is diminishing when the passing cloud obscures the sun. The temperature under field condition does never rise above the maximum temperature limit of activity of this weevil, i.e. 42.92°C, so that the higher is the temperature the more active is the activity of the said

weevil and no cluttering of the activity of the weevil caused by the high temperature is observed.

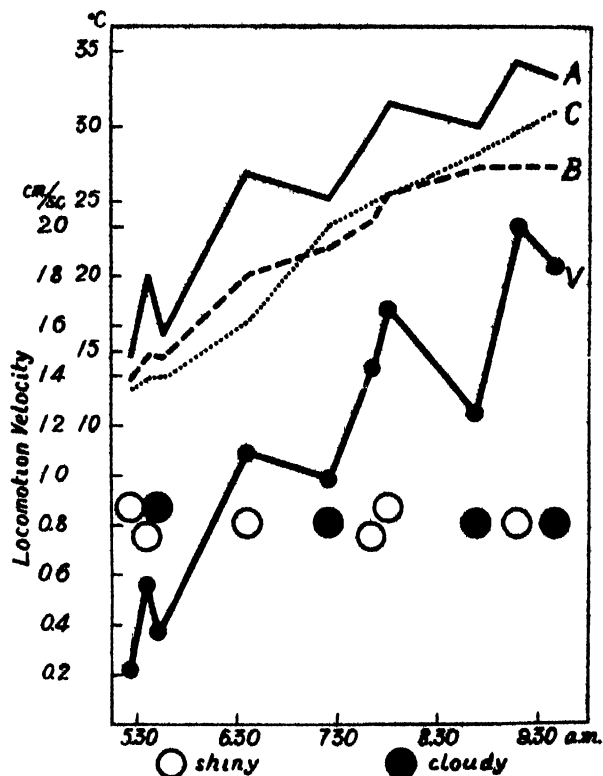


Fig. 2 Correlation between the locomotion velocity of the Strawberry Weevil and the variation of the various temperature factors observed under the field condition. V: Locomotion velocity, A: Solar radiation, B: Air temperature, C: Soil surface temperature.

## DISCUSSION

(1) According to the PARKER's investigation executed on the American Grasshopper, *Melanoplus mexicanus mexicanus* SAUSSURE and *Camnula pellucida* SCUDDER, the air temperature and the soil surface temperature at the time, when the beginning of the activity was noted, were 16.8°C and 26.1°C respectively in the case of the former and 16.7°C and 25.3°C in the latter (PARKER, 1930).

The effect of the physical condition of the environment on the sand

dune insects, especially of the temperature, was clarified by CHAPMAN and others (CHAPMAN, MICKEL, PARKER, MILLER and KELLY, 1926). According to them, the temperature limit of activity is not the same for each insect, which comprises the sand dune insect fauna. For instance, under the field condition *Spharagemon* starts to move about at 13.5°C of the air temperature and 14–19°C of the soil surface temperature, but in the other hand the first *Microbembex* was seen just at 18.5°C of the former temperature and 23°C of the latter one.

In the case of the Strawberry Weevil, the average air temperature, soil surface temperature and reading of the black bulb at the time when the beginning of the crawling was observed were 7.2°C, 7.3°C and 8.4°C respectively.

In these three cases mentioned above, the activity of the Strawberry Weevil begins at the lowest temperature. This fact suggests, that comparing with the grasshoppers and the bembicid, the Strawberry Weevil is an insect adjusting itself to the low temperature.

(II) PARKER observed that the migration of the said grasshopper would alternately start and stop, according to the absence or presence of passing cloud before the sun (PARKER, 1930). Besides, it is very interesting to see that the larval swarms of *Dociosturus maroccanus* TRNBG. start their daily march when the temperature reaches about 23–27°C and stop whenever it drops to the same level, owing to clouds or wind (SIVIRIDENKO, 1903).

It is clearly mentioned that the Strawberry Weevil, which is resting in the place where the sun directly shines, begins to move earlier than the one resting in the shade. Besides, the weak active condition is very often observed in the shade, although the very active condition is already seen in the sunshine. The Strawberry Weevil which begins to crawl, often migrates into the rays of the sun. In addition to these facts it is very noticeable that the difference between the cloudy and fine days is observed in the course of the transformation from the resting nocturnal condition to the active diurnal condition.

According to these observations, it is permissible to conclude that the activities of the Strawberry Weevil exactly follow the temperature reaction. In the case of the Strawberry Weevil, it is very important that the climatic condition, transforming from the night to the morning, is distinctly different between the fine day and cloudy day. In other words, it is conceivable that the solar radiant energy gives an important effect upon the activity of the Strawberry Weevil.

In spite of the sudden decreasing of the solar radiation, the activity, in the afternoon, is gradually weakened. This is easily understood by the fact that the air temperature and the soil surface temperature, being raised by the preceding solar radiation, maintain the effective temperature zone of activity. In paralleling with the gradual lowering of the temperature, the diurnal activity of this weevil passes slowly into the nocturnal condition.

(III) If it is admitted that the general activity follows exactly the temperature reaction, the active weevils may be observed in the night when the temperature is high and is suitable for the activity of the weevil. This is often observed as far as the evaporation or other mechanical factors do not inhibit the said activity; consequently the egg-laying is also recorded. One of these instances is shown in the experiment done from the evening of the 15th of May of 1936 to the morning of the following day (Table 4, Fig. 3).

TABLE 4

*Egg-laying activity of the Strawberry Weevil during the period extending from 6 am. of the 15th of May to 10 am. of the following day.*

Time-interval	Number of eggs	Solar energy	Soil-temperature	Air-temperature	Evaporation
15/May: 6.00-10.00	158	97.9	19.1°C	19.2°C	5.0
10.00-14.00	290	167.8	28.5	28.6	11.9
14.00-18.00	192	58.3	26.1	23.7	5.0
18.00-22.00	60	—	19.5	15.6	2.9
22.00- 2.00	16	—	16.4	12.4	0.7
16/May: 2.00- 6.00	55	—	14.9	11.7	1.4
6.00-10.00	76	22.3	14.8	12.1	2.0

In this case the number of eggs laid was recorded at the interval of four hours. 60 eggs were laid in the experimental field during the period extending from 6 pm. to 10 pm. of the 15th of May, and, as shown by the record obtained at 2 am., 16 eggs were laid in the following four hours of that night. Besides, one female which had been laying an eggs was at that time observed. The above mentioned fact is easily recognized in the following, i. e. the temperature factors were within the effective temperature zone of activity of this weevil. But the fact, that the number

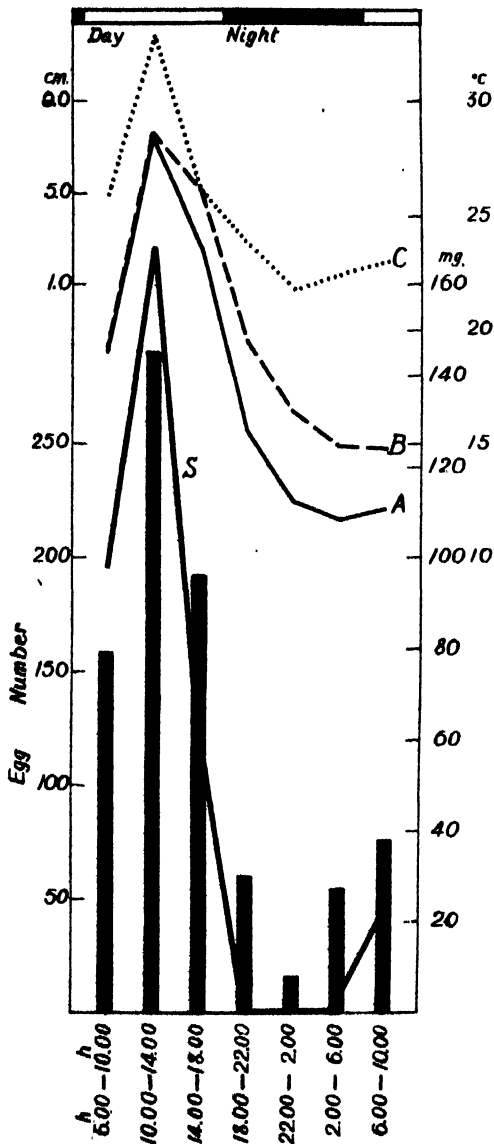


Fig. 3. The egg-laying activity and the variation of various climatic factors observed during the period extending from 6 am. of the 15th of May to 10 am. of the following day. S: Solar energy, A: Air temperature, B: Soil surface temperature, C: Evaporation.

of eggs laid is not so great as expected from the reading of the thermometer, may be explained by the evaporation. The close correlation shown by the evaporation in the night with the egg-laying activity is already seen in my previous paper (1937 a). Accordingly, it was proved that the weevil is active while it is keeping away from the dewpoint. At 2 am. of the 16th of May it was observed that a few water drops were attached to some strawberry plants and one weevil embedded in a water drop attached on a peduncle. But the number of eggs laid during the following four hours reached as many as 55, as shown by the record of 6 am., because the proper evaporation was made and the environmental condition was suitable for the action of the said weevil. Needless to say, no water drops were on the plant at 6 am. Though it is evident, as mentioned above, that the egg-laying is done in the night when the environmental factors are suitable for weevils, the action of the weevil, being inhibited by the mechanical factors

such as water drops or low temperature, can not be generally observed during the night.

Mating condition may also be done in the night under a good climatic condition. This was already recognized by KINOSHITA and SHINKAI (1926).

It is very noticeable that ROCKWOOD (1925) recorded the flight of grasshopper at night when the air temperature was about 26.7°C, and this was not uncommon during warm weather.

(IV) Consequently, it seems to be doubtless that in the case of the Strawberry Weevil the diurnal rhythm of the light intensity of the sun is not so much important for the diurnal activity of the weevil. This is also known by the laboratory experiment. Some Strawberry Weevils and plants were confined in a dark box and reared in a 23°C-thermostat. In this experiment many injured flower-bud oviposited by the weevils were noted, and the active flying was recognized by the sound clashed against the inner side of the box, and, moreover, the crawling trace was recorded on the smoked paper settled in the dark box.

(V) As mentioned above, it is permissible to conclude that the activity of the Strawberry Weevil goes mainly with the temperature reaction. That is to say, the diurnal rhythm of the activity of the said weevil is influenced, first by the temperature factors, such as the solar radiant energy, the air temperature and the soil surface temperature, and second by other various factors.

#### ECOLOGICAL MEANING OF THE SOLAR RADIANT ENERGY

We have learned that the solar radiant energy is most effective upon the egg-laying activity of the Strawberry Weevil (KATÔ, 1937 a, 1937 b) and, moreover, it may be permissible now to extend this opinion to the general activity of the said weevil.

By comparing the reading of the black bulb of the solar radiation thermometer, the air temperature and the soil surface temperature, at which the first individual, under field condition, begins to crawl, with the temperature, at which the weevil begins to crawl under the gradually rising temperature in the laboratory work (KATÔ, 1938), we are able to determine the important effect of the solar radiant energy upon the activity of the weevil. Under the field condition, the air temperature and the soil surface temperature and the reading of the black bulb, at which the beginning of crawling was noted, were 5.0°C, 6.5°C and 7.2°C respectively. Now, in the laboratory work, the weevil can never begin

to crawl under such a temperature as  $6.5^{\circ}\text{C}$ ; consequently, it is very difficult to recognize that the temperature at  $6.5^{\circ}\text{C}$  or  $5.0^{\circ}\text{C}$  brings the beginning of the activity under the field condition. Furthermore, the individuals, resting in the sunshine, begin to crawl earlier than those resting in the shade. It is suggested from the above mentioned that the solar radiant energy gives an influence directly upon the activity of the said weevil and, therefore, the reading of the black bulb, at  $7.2^{\circ}\text{C}$ , represents the exact temperature factor influencing the activity. That the moving weevil was already seen at 4.50 am. on the 18th of May and also at 5 am. on the 20th of the same month is rational, because the reading of the black bulb was above  $10^{\circ}\text{C}$ , which is higher than the lowest limit of the normal activity i. e.  $9.70^{\circ}\text{C}$ .

In the fine cool morning, the weevils, resting in the sunshine, begin to crawl earlier than the other resting in the shade, and the slowly crawling weevils often migrate into the rays of the sun. In the cloudy morning, the resting condition gradually and uniformly changes into the active one. These facts may prove the important effect of the solar radiant energy upon the activity of the weevil. Accordingly, to say the least, in the fine weather, the activity of the Strawberry Weevil depends mainly upon the solar radiant energy in addition to the environmental air temperature. This is, moreover, emphasized by the followings.

Now it is very important to discuss the relation between the locomotion velocity shown in the field experiment and the one obtained in the laboratory experiments. This results is shown in Fig. 4. (A) represents the result obtained by the laboratory work under the gradually rising temperature (KATÓ, 1938). If the weevil crawls under the field condition depending upon the environmental air temperature, the velocity under various temperature is plotted as shown in (B), but if depending upon the solar radiant energy, it is shown in (C). We can see from Fig. 4 that the locomotion velocity was influenced not only by the environmental air temperature, but directly by the solar radiant energy. It is also suggested that the fairly large absorption power of the heat energy may be recognized in the case of the Strawberry Weevil. This fact is also distinctly seen from Table 3 and Fig. 2. It is clearly indicated that the locomotion velocity has a close relation with the reading of the solar radiation thermometer. It was already mentioned that the climbing velocity is diminished when passing clouds obscure the sun. It seems that this depends exactly upon the sudden decreasing of the solar energy as known from the reading of the black bulb. Consequently, the air tem-



perature and the soil surface temperature, which are not influenced by passing clouds, are not so much noticeable. Besides, the comparison of

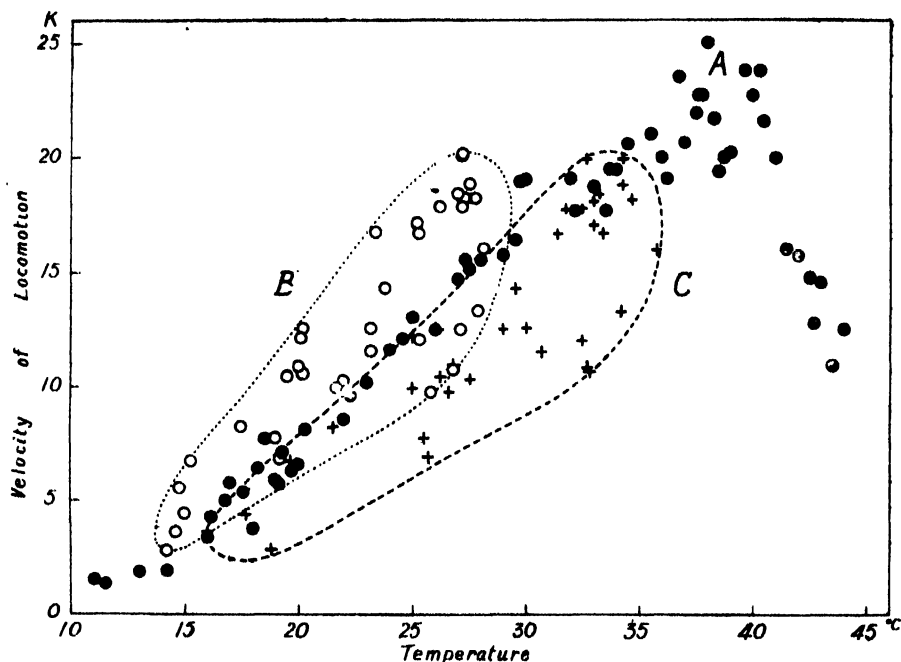


Fig. 4. Locomotion velocity of the Strawberry Weevil measured under the field condition and in the laboratory.

the temperature at which the beginning of the flying, measured under the field condition with that obtained by the laboratory work, is very important. Under the field condition the first flying begins at 18°C of the air temperature and 24.8°C of the black bulb, when, in the laboratory work, it is at 22.8°C. It is easily conceivable that under the field condition the first flying is not brought by the air temperature, but directly by the influence of the solar radiant energy. Actually, in the laboratory work, the flying is never seen under such condition when the environmental temperature is 18°C.

The solar radiant energy has never been recognized as one of the environmental factors in the field of ecology. Nevertheless, this is actually important as already mentioned. The fact that most of the diurnal insects are very active in the fine weather and that this depends mainly upon the temperature factors is generally well known. But it seems that the

solar radiant energy has not ever almost been taken up as one of the temperature factors.

Considering from the present ecological investigation made on the activity of the Strawberry Weevil, it seems to be very important to take the solar radiant energy as one of the temperature factors. The investigation on the body temperature executed in the case of grasshopper by BODENHEIMER (1930) seems to suggest the above mentioned idea. Moreover, this idea may also give an important temperature factor to the opinion expressed by UVAROV (1928), i. e. the effect of the environmental temperature is not given directly upon the activity of the insect, but indirectly through the body temperature.

#### SUMMARY

1. In the present paper, the diurnal activity of the Strawberry Weevil, *Anthonomus bisignifer* SCHENKLING, especially the process transforming from the nocturnal resting condition to the diurnal active condition, was dealt with, and at the same time, the relation between the said activity and the temperature factors was investigated.

2. Under the field condition the Strawberry Weevil begins to move and transforms from the resting condition into the active one when the reading of the black bulb of the solar radiation thermometer, the air temperature and the soil surface temperature are 8.4°C, 7.2°C and 7.3°C respectively.

3. In the cloudy weather the said transformation progresses uniformly and gradually all over the strawberry plants. But, on the contrary, in the fine weather the progression is not uniform; the individual resting in the sunshine begins to crawl earlier than the others resting in the shade.

4. When the air temperature and the reading of the black bulb of the solar radiation thermometer rise up to 18°C and 24.8°C, the beginning of the flying is noted. The weevil which was crawling in the sunshine begins to fly first.

5. It is very often observed and noticeable that the locomotion velocity diminishes when the passing cloud obscure the sun.

6. The diurnal rhythm of the activity of the said weevil seems to depend mainly upon the diurnal variation of the temperature factors, such as the air temperature, the soil surface temperature and the solar radiant energy. The effect of the solar radiant energy given upon the

activity of weevil in the daytime is very noticeable. In the unusually warm night the weevil is active as in the daytime.

7. Finally, the meaning of the solar radiant energy in the ecological investigation was discussed. It may be conceivable that the solar radiant energy which has ever been neglected is a very important temperature factor, as in the cases of the air temperature and the soil surface temperature.

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# PROTEOLYTIC ENZYMES IN A SEA ANEMONE, *CRIBRINA ARTEMISIA* (DANA)

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(With eight figures)

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Many biologists had been interested in the digestive enzymes of coelenterates from old times (HOLLARD 1851, 1854; LEWES 1859). As to the digestive processes in actinians, METSCHNIKOFF (1880) first reported that the intracellular digestion takes place in the cells lining the mesenteric filament of *Sagartia* and *Aiptasia* from his experiments with carmine. KRUKENBERG (1881, 1882) concluded that digestion occurs at the surface of contact between the filament and the food in actinia. CHAPEAUX (1893) was in agreement with KRUKENBERG, but recognized, nevertheless, the presence of weak proteolytic enzymes in the gastric fluid of *Sagartia*, *Anemonia*, and *Heliactis*.

MESNIL's observations (1893) showed that digestion in actinia is not so much dependent upon the contact of the filaments with the food as upon the absorption of the food stuffs within the amoeboid cells lining the mesenteric filaments. JORDAN (1907) reasserts that slight digestion occurs in the gastric cavity of actinia, *Anemonia sulcata*. BODANSKY (1924) concluded that in *Metridium* the digestive enzymes occur, for the most part, intracellularly. However, partial disintegration of foods may take place within the digestive cavity as a result of autolytic changes and to some extent by extracellular enzymes having their origin in the cells lining the mesenteric filaments. Recently, ISHIDA (1936) reported that in *Actinia mesembryanthemum* the extracellular digestion takes place with the aid of much slime.

FRÉDÉRICQ (1878) reported the presence of a trypsin-like enzyme in mesenteric filament extract of actinians. KRUKENBERG (1881) confirmed the presence of a tryptic enzyme in the glycerine extract of mesenteric filament of actinians and the presence of an enzyme to digest the raw fibrin in the glycerine or water extracts of the mesenteric filament of *Sagartia* and *Anthea*. And also he reported the presence of an active

peptic enzyme to digest the raw fibrin completely at 38°C in presence of 0.2 per cent HCl within  $\frac{1}{2}$  to 1 hour in the water extract of the mesenteric filament of *Cerianthus cylindricus*, but denied the presence of a tryptic enzyme in that extract of this form. CHAPEAUX (1893) states that the albuminoid was digested to peptone by the cell elements of the gastric cavity, and points out the presence of a protease (tryptic enzyme) in the mesenteric filament extract of actinians which digests the fibrin in alkaline or very slightly acid medium which is inactive in strong acid one. Finding tyrosine in the digestive products by the mesenteric filament extract of *Adamsia rondeletti*, MESNIL (1901) confirmed the presence of a protease in that extract.

ABDERHALDEN and HEISE (1909) detected peptolytic enzyme (polypeptidase) in *Actinia equina* by using silk-peptone (glycyl-l-tyrosine) as substrate. BODANSKY (1924) showed that in the mesenteric filament extract of *Metridium marginatum* pepsin is present in but minute traces and trypsin is the predominant proteolytic enzyme. SAWANO (1931) reported the presence of a protease to digest gelatine and peptone at optimum pH 6.9 and 7.9 respectively in the gastric filament of sea anemone, *Actinodendron*. Finding tyrosine and tryptophane in the digestive products by the sea water extract of mesenteric filament of *Adamsia rondeletti* or *Anemonia sulcata*, ABDERHALDEN (1932) confirmed the presence of a tryptase in that extract.

KRUKENBERG (1881) reported the presence of a peptic enzyme in the tentacle extract of *Actinia mesembryanthemum*. MESNIL (1901) denied the presence of a protease in the tentacle extract of *Adamsia rondeletti*. MESNIL (1901) could not detect protease in the extract of the oesophagus of *Adamsia rondeletti*. So far as I am aware, there is no precise information with regard to the enzymes in the stomodaeum of actinians.

CHAPEAUX (1893) recognized the presence of a weak proteolytic enzyme in the gastric fluid of *Sagartia*, *Anemonia* and *Heliactis*. JORDAN (1907) reports that slight digestion takes place in the gastric cavity of *Anemonia sulcata*. BODANSKY (1924) showed that the fluid removed from the gastro-vascular cavity of *Metridium marginatum*, after filtration, exerts no measurable digestive effect on proteins. On the other hand, such fluid, if unfiltered, is capable of digesting gelatine in neutral or slightly alkaline solutions. Quite recently, ISHIDA (1936) confirmed the presence of tryptase, pepsinase and ereptase (dipeptidase) as proteolytic enzymes not only in the unfiltered gastric fluid but also in the filtered gastric fluid of *Actinia mesembryanthemum* and reported that the enzymes in the gastric fluid

have the origin from the cells lining the mesenteric filament.

In the present paper I have reported the experiment on the proteolytic enzymes in *Cribrina artemisia* (DANA), which was collected near the Marine Biological Station, Asamushi, Aomori-Prefecture. The experiment was carried on at the Biological Institute of Tôhoku Imperial University at Sendai.

Here I must express my heartiest thanks to Prof. S. HATAI and Dr. T. KOIZUMI for their kind guidance throughout the course of this work, and to Prof. S. HÔZAWA who gave me anatomical advices, and also to Prof. T. UCHIDA of the Hokkaidô Imperial University for his kind identification of the actinian.

## EXPERIMENTS

### The Enzyme Solution

The actinians were killed 4 days after capture during which time no food was given. Gastric fluid was taken by cutting the base of actinians with scissors, or, by inserting a spoit into the gastric cavity, care being taken not to injure the filament. The fluid was then put into an Erlenmeyer's flask, adding chloroformtoluol mixture (1:1) as an antiseptic. The filtrate through four sheets of gauze is "unfiltered gastric fluid" and the filtrate through filter paper "filtered gastric fluid". Mesenteric filaments, tentacles and stomodaeum were freed from foreign tissues, washed with sea water, and weighed after removing the water by filter paper. Then ground in a mortar to paste with quartz sand, and mixed well with the 87 per cent glycerine of 3 or 5 times its weight. The glycerine extracts were kept in Erlenmeyer's flask with a stopper, adding few drops of antiseptic to prevent putrefaction, and placed in a dark place at room temperature for 3 days and centrifuged. The supernatant fluids, with antiseptic, were kept in the ice-box and diluted twice or thrice with distilled water before use.

The numbers of whole body and the weight of materials for enzyme solution in six instances were as follows:

No. of body	m.f. gr.	Tentacle gr.	stom. gr.	Fluids	
				(unfiltered+filtered)	
				cc.	
43	5.4	2.3	3.2	ca.	44
52	4.67	2.4	2.56	"	30
36	6.35	0.15	3.65	"	25
34	7.86	3.35	6.82	"	25
63	7.2	0.7	9.7	"	70
55	5.0	1.42	9.8	"	60
Sum 283	36.48	10.32	35.73		254

Substrates used are as follows: Gelatine after Dr. G. GRÜBLER; n-chloracetyl-l-tyrosine after Drs. FRAENKEL and LANDAU; dl-leucyl-glycyl-glycine after Drs. FRAENKEL and LANDAU; glycyl-glycine after Dr. FRAENKEL.

### Method of the Estimation of the Proteolytic Activity

Digestivity was estimated by the ordinary Willstätter's titration method, and thymolphthalein was used as the indicator. The titration was carried on as follows: 0.5 cc. of digestive mixture is poured into a test-tube and 0.5 cc. of 0.1 per cent indicator in absolute alcohol and 4 cc. absolute alcohol are added, and titrated with micro-burette by 0.02439 N (or 0.03252 N or ca. 0.04 N) KOH in 90 per cent alcohol to a distinct coloration stirring with CO<sub>2</sub>-free air bubbling in the dark room. pH of the digestive mixtures was thus estimated: 4 or 5 cc. of the mixture were centrifuged and the supernatant fluid itself or it diluted twice with about 0.1 M NaCl solution was colorimetrically estimated, before or after the digestion.

Note 1. A drop of antiseptic is added invariably in every case.

Note 2. Control experiments were set parallel with the boiled enzymes and negative results were obtained.

### Detection and Optimum pH of the Proteolytic Enzymes in the Various Sources

The digestion of gelatine, dl-leucyl-glycyl-glycine, n-chloracetyl-l-tyrosine and glycyl-glycine are tested in various sources such as, mesenteric filament, stomodaeum, tentacle, unfiltered and filtered gastric fluid, by the methods just stated and the results obtained are shown in Tables 1, 3, 5, 9, 11 and 13, Figs. 2 and 3.

The effects of pH on the digestive activity of the enzymes were investigated in the extracts of mesenteric filament and stomodaeum and the results obtained are shown in the following tables and figures (Tables 2, 4, 6, 7, 8, 10, 12 and 14, Figs. 1, 4, 5, 6, 7 and 8).

## RESULTS

### 1. *Pepsinase*

#### a) Detection of Pepsinase.

The procedure and results are shown in Table 1.

TABLE 1.  
*Detection of Pepsinase.*

No.	Samples	0.02439 N KOH in cc.						
		Time 0	After 1 day	Increase after 1 day	After 5 days	Increase after 5 days	After 10 days	Increase after 10 days
1	Mesenteric filament extract	1.31	1.32	0.01	1.37	0.06	1.50	0.19
		Cont. 1.23	1.23	0.00	1.24	0.01	1.24	0.01
2	Stomodaeum extract	1.18	1.18	0.00	1.18	0.00	1.18	0.00
		Cont. 1.15	1.15	0.00	1.15	0.00	1.15	0.00
3	Tentacle extract	1.17	1.17	0.00	1.17	0.00	1.17	0.00
		Cont. 1.17	1.17	0.00	1.17	0.00	1.17	0.00
4	Unfiltered gastric fluid	1.14	1.14	0.00	1.18	0.04	1.21	0.07
		Cont. 1.15	1.15	0.00	1.15	0.00	1.15	0.00
5	Filtered gastric fluid	1.12	1.12	0.00	1.18	0.06	1.20	0.08
		Cont. 1.09	1.09	0.00	1.10	0.01	1.10	0.01

1 cc. enzyme solution+2 cc. buffer solution (Sørensen's M/10 citrate-N/10 HCl, pH 1.418)+1 cc. 3% gelatine solution. Kept for 10 days at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

It shows that pepsinase is absent in the extracts of tentacle and stomodaeum but found in other sources.

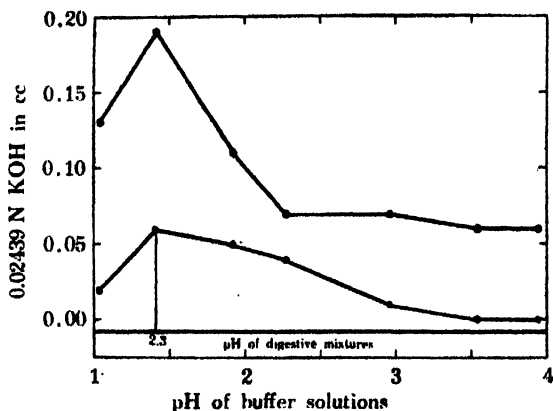
b) Optimum pH of Pepsinase.

TABLE 2.  
*Effect of pH on the activity of pepsinase.*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C			0.02439 N KOH in cc.						
			Time 0	After 1 day	After 5 days	Time 0	After 1 day	Increase after 1 day	After 5 days	Increase after 5 days	After 10 days	Increase after 10 days
1	Citrate-HCl	1.038				1.41	1.41	0.00	1.43	0.02	0.54	0.13
2	..	1.418	2.27		2.3	1.31	1.32	0.01	1.37	0.06	1.50	0.19
3	..	1.925	2.8	2.8		1.30	1.32	0.02	1.35	0.05	1.41	0.11
4	..	2.274				1.29	1.31	0.02	1.33	0.04	1.36	0.07
5	..	2.972				1.35	1.35	0.00	1.36	0.01	1.42	0.07
6	..	3.529				1.33	1.33	0.00	1.33	0.00	1.39	0.06
7	..	3.948				1.31	1.31	0.00	1.31	0.00	1.37	0.06

1 cc. enzyme solution+2 cc. buffer solution of varying pH value+1 cc. 3% gelatine solution. Kept for 10 days at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.





• Titration of 5-day samples: ○ Titration of 10-day samples

Fig. 1. Showing the pH optimum curve of pepsinase in the mesenteric filament extract, based on Table 2.

As seen in Table 2 and Fig. 1, the optimum reaction of pepsinase in the mesenteric filament on gelatine was 2.3.

## 2. Cathepsin

### a) Detection of Cathepsin.

TABLE 3.  
*Detection of cathepsin.*

		0.03252 N KOH in cc.				
No.	Samples	Time 0	After 1 day	Increase after 1 day	After 3 days	Increase after 3 days
1	Mesenteric filament extract	1.21 Cont. 0.98	1.26 1.00	0.05 0.02	1.37 1.02	0.16 0.04
2	Stomodaeum extract	1.15	1.16	0.01	1.16	0.01
3	Tentacle extract	1.16 Cont. 1.00	1.24 1.00	0.08 0.00	1.30 1.00	0.14 0.00
4	Unfiltered gastric fluid	1.03	1.03	0.00	1.03	0.00
5	Filtered gastric fluid	1.05	1.05	0.00	1.05	0.00

0.5 cc. enzyme solution + 0.5 cc. 1% cysteine hydrochloride solution + 2 cc. buffer solution (M/5  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ -M/10 citric acid, pH 5.0) + 2 cc. 3% gelatine solution.\* For the control experiment  $\text{H}_2\text{O}$  instead of cysteine hydrochloride solution is added. They are kept for 3 days at  $37^\circ\text{C}$ . 0.5 cc. of digestive mixture is titrated by Willstätter's method. \* pH of the resulting solution was found to be 4.8.

Effect of cysteine hydrochloride solution on the proteolytic activity of the extracts of various sources is examined. The method and results are shown in Table 3, Figs. 2 and 3.

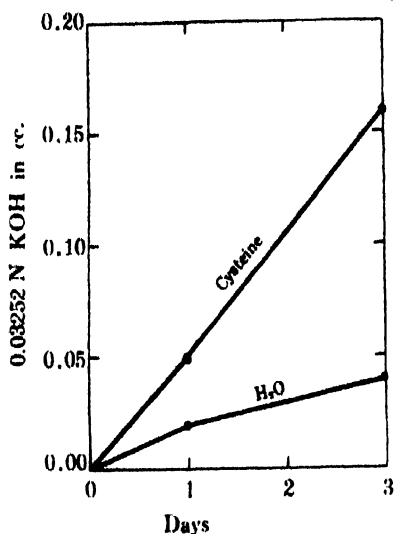


Fig. 2. Showing the effect of cysteine hydrochloride solution on gelatine digestion by the extract of mesenteric filament, based on Table 3.

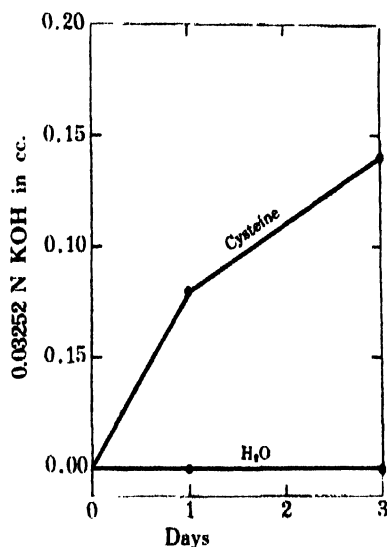


Fig. 3. Showing the effect of cysteine hydrochloride solution on gelatine digestion by the extract of tentacle, based on Table 3.

Gelatine digestion by the extracts of mesenteric filament and tentacle is accelerated obviously by the presence of cysteine hydrochloride solution at about pH 5.0, this fact proves the presence of some cathepsin-like enzyme in these sources.

#### b) Optimum pH of Cathepsin.

Effect of cysteine hydrochloride solution on the pH optimum curve for the digestion of gelatine by the extract of mesenteric filament is shown in Table 4 and Fig. 4. They show that its optimum reaction is 4.8.

TABLE 4.  
*Effect of pH on the activity of cathepsin.*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C			0.03252 N KOH in cc.				
			Time 0	After 1 day	After 3 days	Time 0	After 1 day	Increase after 1 day	After 3 days	Increase after 3 days
1	$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ -citric acid	4.6				1.42	1.43	0.01	1.52	0.10
						Cont. 1.18	1.18	0.00	1.18	0.00
2	"	5.0	4.8	4.8	4.75	1.23	1.25	0.02	1.39	0.16
						Cont. 0.98	1.00	0.02	1.02	0.04
3	"	5.6				1.03	1.03	0.00	1.12	0.09
						Cont. 0.85	1.06	0.21	1.23	0.38
4	"	6.0				0.96	0.96	0.00	1.01	0.05

0.5 cc. enzyme solution+0.5 cc. 1% cysteine hydrochloride solution+2 cc. buffer solution of varying pH value+2 cc. 3% gelatine solution. For the control experiments  $\text{H}_2\text{O}$  instead of cysteine hydrochloride solution is added. They are kept for 3 days at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

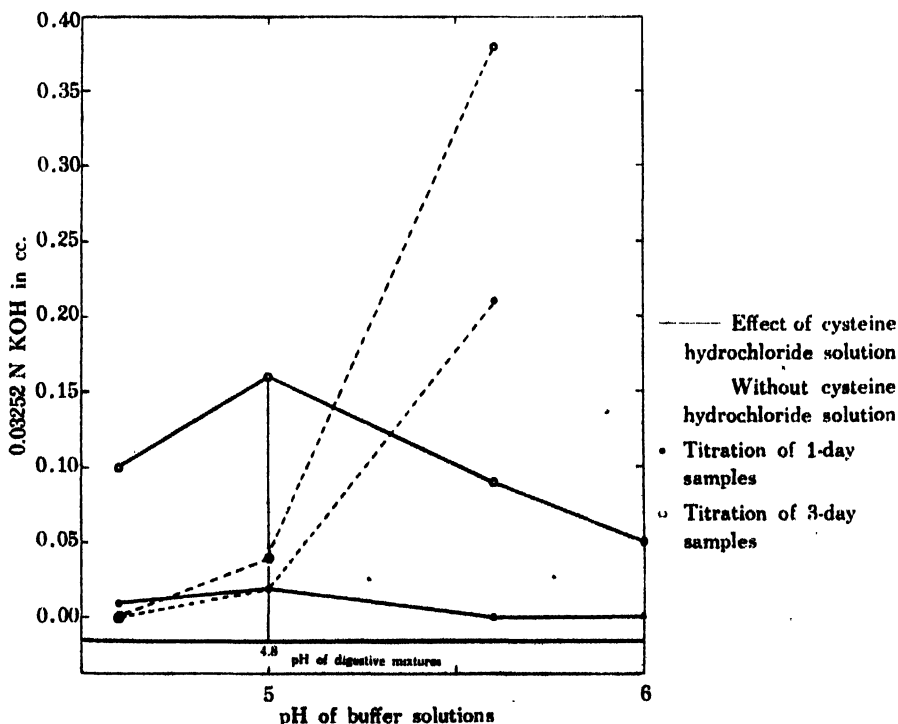


Fig. 4. Showing the pH optimum curve of cathepsin in the mesenteric filament extract with or without cysteine hydrochloride solution, based on Table 4.

### 3. Trypsase

#### a) Detection of trypsin.

Table 5 shows that trypsin is found in the extracts of mesenteric filament and stomodaeum and unfiltered gastric fluid.

TABLE 5.  
*Detection of trypsin.*

No.	Samples	0.02439 N KOH in cc.							
		Time 0	After 1 day	Increase after 1 day	After 3 days	Increase after 3 days	After 4 days	After 10 days	Increase after 10 days
1	Mesenteric filament extract	1.30 Cont. 1.20	1.86 1.20	0.56 0.00	1.91 1.20	0.61 0.00			
2	Stomodaeum extract	1.02 Cont. 1.03	1.13 1.03	0.11 0.00	1.20 1.03	0.18 0.00			
3	Tentacle extract	1.01 Cont. 1.00	1.01 1.00	0.00 0.00	1.02 1.00	0.01 0.00			
4	Unfiltered gastric fluid	1.53 Cont. 1.42	1.53 1.42	0.00 0.00			1.54 1.42	1.59 1.42	0.06 0.00
5	Filtered gastric fluid	1.51 Cont. 1.48	1.51 1.48	0.00 0.00			1.52 1.48	1.54 1.48	0.03 0.00

1 cc. enzyme solution + 2 cc. buffer solution (M/10 glycoll--M/10 NaCl--N=0 NaOH, pH 8.52) + 1 cc. 3% gelatine solution. Kept for 10 days at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

Note: In both unfiltered and filtered gastric fluids pH 8.18 (glycoll-NaCl-NaOH) instead of pH 8.52 was employed.

#### b) Optimum pH of trypsin in the mesenteric filament extract.

TABLE 6.  
*Effect of pH on the activity of trypsin (phosphate buffer).*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C		0.02439 N KOH in cc.		
			Time 0	After 1 day	Time 0	After 1 day	Increase
1	Na <sub>2</sub> HPO <sub>4</sub> ·KH <sub>2</sub> PO <sub>4</sub>	5.589			0.91	1.23	0.32
2	"	5.906			0.92	1.25	0.33
3	"	6.468	6.35	6.25	0.81	1.30	0.49
4	"	6.979			0.75	1.21	0.46
5	"	7.381			0.67	1.12	0.45
6	"	8.043			0.62	1.01	0.39

1 cc. enzyme solution + 2 cc. buffer solution of varying pH value + 1 cc. 3% gelatine solution. Kept for 1 day at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

As shown in Tables 6, 7 and 8 and Fig. 5, the optimum reaction of tryptase in the mesenteric filament on gelatine was found to be 6.3 (phosphate buffer), 7.0~6.75 (glycocoll buffer) and 7.3 (borax-boric acid buffer).

TABLE 7.

*Effect of pH on the activity of tryptase (glycocoll buffer).*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C.		0.02439 N KOH in cc.		
			Time 0	After 1 day	Time 0	After 1 day	Increase
1	Glycocoll-NaCl-NaOH	7.5			1.35	1.74	0.39
2	"	8.18	7.0	6.75	1.27	1.80	0.53
3	"	8.52			1.17	1.69	0.52
4	"	8.92			1.00	1.45	0.45
5	"	9.66			0.57	0.99	0.42

1 cc. enzyme solution+2 cc. buffer solution of varying pH value+1 cc. 3% gelatine solution. Kept for 1 day at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

TABLE 8.

*Effect of pH on the activity of tryptase (borax-boric acid buffer).*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C.		0.02439 N KOH in cc.				
			Time 0	After 1 day	Time 0	After 1 day	Increase after 1 day	After 46 hrs.	Increase after 46 hrs.
1	Borax-boric acid	6.77			2.65	2.73	0.08		
2	"	7.09			2.60	2.76	0.16	2.81	0.21
3	"	7.60			2.45	2.75	0.30	2.83	0.38
4	"	7.94			2.29	2.67	0.38	2.73	0.44
5	"	8.51	7.3	7.3	1.98	2.41	0.43	2.48	0.50
6	"	8.98			1.63	2.08	0.45	2.13	0.50
7	"	9.24			1.41	1.82	0.41	1.88	0.47

1 cc. enzyme solution+2 cc. buffer solution of varying pH value+1 cc. 3% gelatine solution Kept for 46 hrs. at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

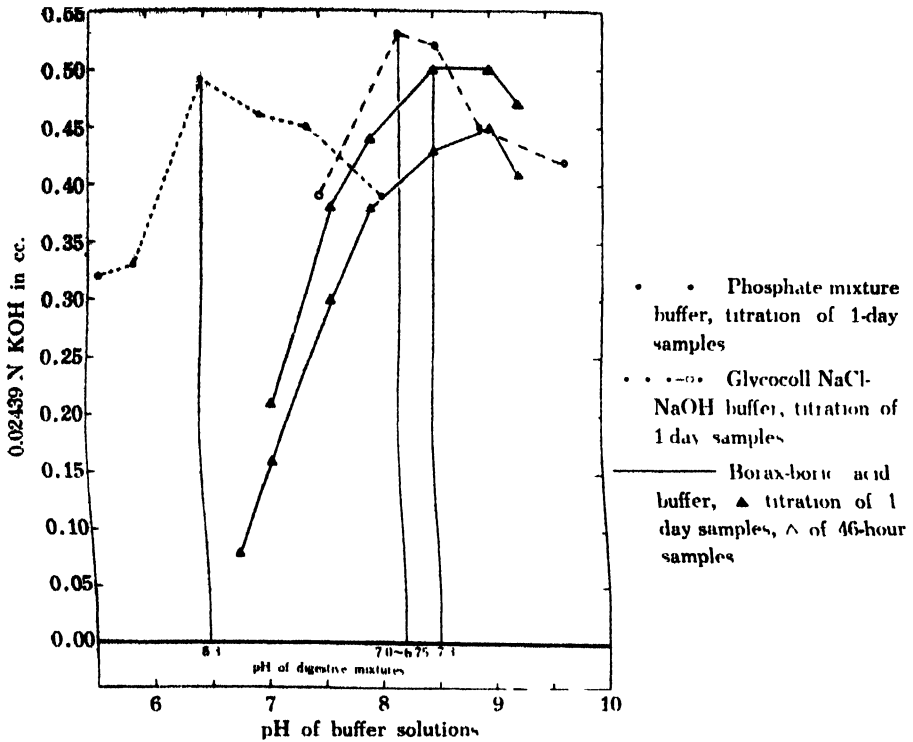


Fig. 5. Showing variation of pH-effect due to different buffers for the pH optimum curve of trypsin in the mesenteric filament extract, based on Tables 6, 7 and 8.

#### 4. Amino-polypeptidase

##### a) Detection of amino-polypeptidase.

TABLE 9.  
*Detection of amino-polypeptidase.*

No.	Samples	0.04 N KOH in cc.				
		Time 0	After 1 day	Increase after 1 day	After 4 days	Increase after 4 days
1	Mesenteric filament extract	0.38	0.43	0.05	0.54	0.16
2	Stomodaeum extract	0.39	0.43	0.04	0.50	0.11
3	Tentacle extract	0.39	0.39	0.00	0.41	0.02
4	Unfiltered gastric fluid	0.42	0.42	0.00	0.46	0.04
5	Filtered gastric fluid	0.43	0.43	0.00	0.46	0.03

1 cc. enzyme solution+2 cc. buffer solution (M/15 phosphate mixtures, pH 7.877)+1 cc. 2% dl-leucyl-glycyl-glycine+0.1 N NaOH 0.05 cc. Kept for 4 days at 37°C. 0.5 cc of digestive mixture is titrated by Willstätter's method.

Table 9 shows that amino-polypeptidase is found in the extracts of mesenteric filament and stomodaeum.

b) Optimum pH of amino-polypeptidase in the mesenteric filament extract.

TABLE 10.  
*Effect of pH on the activity of amino-polypeptidase.*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C (Time 0)	0.04 N KOH in cc.		
				Time 0	After 1 day	Increase
1	Glycocoll-NaCl-NaOH	8.18		1.07	1.07	0.00
2	"	8.52		1.01	1.05	0.04
3	"	8.92		0.84	0.96	0.12
4	"	9.66	8.6	0.58	0.72	0.14
5	"	9.99	8.7	0.52	0.68	0.16
6	"	10.54	8.75	0.46	0.61	0.15
7	"	11.02		0.40	0.53	0.13

1 cc. enzyme solution + 2 cc. buffer solution of varying pH value + 1 cc. 2% dl-leucyl-glycyl-glycine solution. Kept for 1 day at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

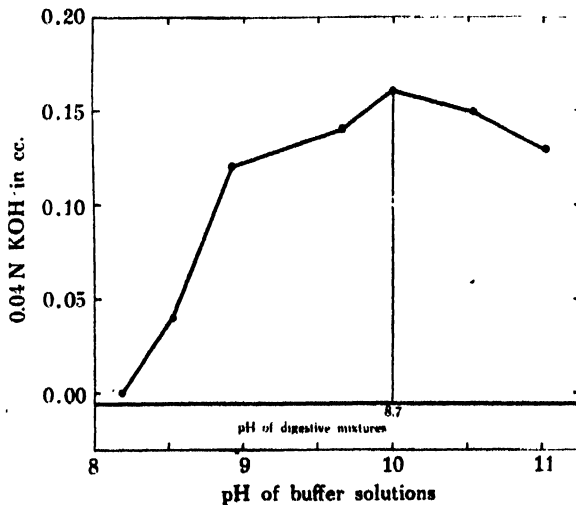


Fig. 6. Showing the pH optimum curve of digestion of dl-leucyl-glycyl-glycine by the mesenteric filament extract, based on Table 10.

As seen in Table 10 and Fig. 6 the optimum pH of amino-polypeptidase

in the mesenteric filament was 8.7.

### 5. Carboxy-polypeptidase

a) Detection of Carboxy-polypeptidase.

The procedure and results are shown in Table 11.

It shows that carboxy-polypeptidase is proved only in the mesenteric filament extract.

TABLE 11.  
*Detection of carboxy-polypeptidase.*

No.	Samples	0.04 N KOH in cc.				
		Time 0	After 1 day	Increase after 1 day	After 3 days	Increase after 3 days
1	Mesenteric filament extract	0.46	0.55	0.09	0.58	0.12
2	Stomodaeum extract	0.44	0.45	0.01	0.45	0.01
3	Tentacle extract	0.44	0.44	0.00	0.45	0.01
4	Unfiltered gastric fluid	0.62	0.63	0.01	0.62	0.00
5	Filtered gastric fluid	0.62	0.62	0.00	0.62	0.00

1 cc. enzyme solution+2 cc. buffer solution (M/10 glycocoll-M/10 NaCl-N/10 NaOH, pH 9.99)+1 cc. 2% n-chloracetyl-l-tyrosine. Kept for 3 days at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

b) Optimum pH of carboxy-polypeptidase in the mesenteric filament extract.

Table 12 and Fig. 7 show that optimum pH of carboxy-polypeptidase was 8.15.

TABLE 12.  
*Effect of pH on the activity of carboxy-polypeptidase.*

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C (Time 0)	0.04 N KOH in cc.		
				Time 0	After 1 day	Increase
1	Glycocoll-NaCl NaOH	9.66	8.15	0.62	0.68	0.06
2	"	9.99		0.53	0.63	0.10
3	"	10.54		0.45	0.54	0.09
4	"	11.53		0.34	0.42	0.08

1 cc. enzyme solution+2 cc. buffer solution of varying pH value+1 cc. 2% n-chloracetyl-l-tyrosine. Kept for 1 day at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.



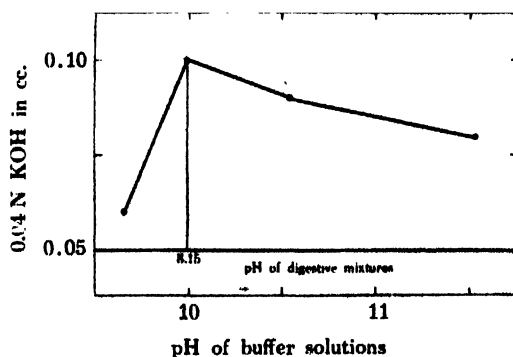


Fig. 7. Showing the pH optimum curve of digestion of n-chloroacetyl-L-tyrosine by the mesenteric filament extract, based on Table 12.

## 6. Dipeptidase

### a) Detection of dipeptidase.

The procedure and results are shown in Table 13. Dipeptidase was detected in the extracts of mesenteric filament and stomodaeum.

TABLE 13.  
*Detection of dipeptidase.*

No.	Samples	0.04 N KOH in cc.				
		Time 0	After 1 day	Increase after 1 day	After 4 days	Increase after 4 days
1	Mesenteric filament extract	0.39	0.52	0.13		
2	Stomodaeum extract	0.45	0.82	0.37		
3	Tentacle extract	0.42	0.42	0.00	0.43	0.01
4	Unfiltered gastric fluid	0.43	0.43	0.00	0.43	0.00
5	Filtered gastric fluid	0.45	0.45	0.00	0.45	0.00

1 cc. enzyme solution + 2 cc. buffer solution (M/15 phosphate mixtures, pH 7.825) + 1 cc. 2% glycyl-glycine + 0.4 cc. N/10 NaOH. Kept for 4 days at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.

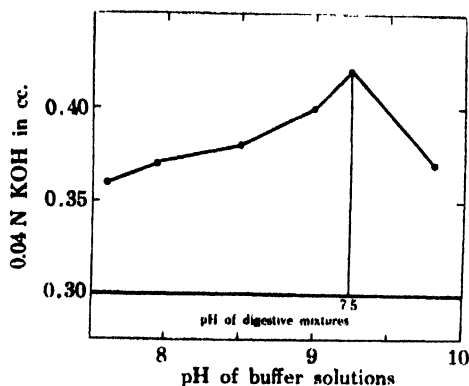
### b) Optimum pH of dipeptidase in the stomodaeum extract.

As seen in Table 14 and Fig. 8, the optimum reaction of dipeptidase was 7.5.

TABLE 14.  
Effect of pH on the activity of the dipeptidase.

No.	Buffer solutions	pH of buffer solutions	pH of digestive mixtures at 37°C.		0.04 N KOH in cc.		
			Time 0	After 1 day	Time 0	After 1 day	Increase
1	Borax-boric acid	7.60			1.74	2.10	0.36
2	"	7.94			1.68	2.05	0.37
3	"	8.51			1.54	1.92	0.38
4	"	8.98			1.35	1.75	0.40
5	"	9.24	7.5	7.5	1.19	1.61	0.42
6	"	9.80			0.95	1.32	0.37

1 cc. enzyme solution + 2 cc. buffer solution of varying pH value + 1 cc. 2% glycyl-glycine. Kept for 1 day at 37°C. 0.5 cc. of digestive mixture is titrated by Willstätter's method.



Summarizing the above results, the following tables are obtained (Tables 15 and 16).

Fig. 8. Showing the pH optimum curve of digestion of glycyl-glycine by the stomodaeum extract, based on Table 14.

TABLE 15.

Showing the distribution of fractional enzymes in the various sources in *Cribrina artemisia* (DANA).

	Mesenteric filament extract	Stomodaeum extract	Tentacle extract	Unfiltered gastric fluid	Filtered gastric fluid
Pepsinase	+	—	—	trace	trace
Cathepsin	+	—	+	—	—
Trypsin	++	+	—	trace	—
Amino-polypeptidase	++	+	—	—	—
Carboxy-polypeptidase	+	—	—	—	—
Dipeptidase	+	++	—	—	—

The sign + denotes that digestion is proved and ++ denotes the efficiency of the action, the sign — denotes that no effect has so far been observed.

TABLE 16.

*Optimum pH of proteolytic enzymes found in the mesenteric filament and stomodaeum of Cribrina artemisia (DANA).*

Enzymes	Mesenteric filament extract	Stomodaeum extract
Pepsinase (Gelatine as the substrate)	2.3	
Cathepsin (Gelatine as the substrate)	4.8	
Tryptase (Gelatine as the substrate)	6.3, 7.0~6.75 & 7.3	
Amino-polypeptidase (dl-leucyl-glycyl-glycine as the substrate)	8.7	
Carboxy-polypeptidase (n-chloracetyl-l-tyrosine as the substrate)	8.15	
Dipeptidase (Glycyl-glycine as the substrate)		7.5

## DISCUSSION

MESNIL (1901) reported that among the extracts of mesenteric filament, tentacle, oesophagus and acontins in *Anemonia sulcata* and *Adamsia ron-deletti* the mesenteric filament extracts have the largest quantity of enzymes. Similar results were also obtained in *Cribrina artemisia* (DANA).

BODANSKY and ROSE (1922) and BODANSKY (1924) said that in three species of coelenterates (*Physalia*, *Stomolophus* and *Metridium*) pepsin

TABLE 17.

*Comparison of optimal reaction of pepsinase and tryptase in the mesenteric filament of various actinians.*

Materials	Optimal reaction		Methods	Authors
	Pepsinase	Tryptase		
<i>Metridium</i>	3.0, 3.5	7.3, 7.6	Gelatine liquefaction	BODANSKY and ROSE (1922) and BODANSKY (1924)
<i>Actinodendron</i>		6.9	Willstätter's titration*	SAWANO (1931)
<i>Actinia mesembryanthemum</i>	1.17	8.88, 9.98	Gelatine liquefaction and micro-Van Slyke*	ISHIDA (1936)
<i>Cribrina artemisia</i> (DANA)	2.2~2.3	6.3, 7.0~6.75, 7.3	Willstätter's titration*	TAKEMURA (1938)

\* Gelatine was used as the substrate.

digests gelatine most rapidly at pH from 3.0 to 3.5. Likewise trypsin manifests maximum effect in an alkalinity equivalent to 7.3 to 7.6 and is the predominant proteolytic enzyme (Table 17).

And also according to SAWANO (1931) a protease was detected in the extract of gastric filament in a sea anemone, *Actinodendron* which digests gelatine at optimum pH 6.9. ISHIDA (1936) confirmed the presence of two proteinases (pepsinase and tryptase) in the mesenteric filament extract and gastric fluid of *Actinia mesembryanthemum* which digests gelatine at optimum pH about 1.17 (citrate-HCl), 8.88 (ammonia-NH<sub>4</sub>Cl buffer) and 9.98 (borax-NaOH buffer), but the optimum pH values seem to be not pH values of the digesting mixture but of the buffer solution (Table 17).

Recently SAVIANO (1937) investigated a hydrolysis of casein by the glycerine extract of endoderm of *Anemonia sulcata* at pH 2 (citrate-HCl), 6.7 (citrate-NaOH buffer) and 8.9 (NH<sub>4</sub>OH-NH<sub>4</sub>Cl buffer) by the ordinary methods, and he said that the extract, as seen in the titrations of 2~24 hours samples, contains a proteinase resembling trypsin in being more active in an alkaline medium but no or minute proteinase resembling pepsin. The results, which I have obtained in the present investigation, are about similar to the experiments of those authors, except that in regard to the optimal hydrogen ion concentrations there are some differences (Table 17); that is according to my experiments pepsinase, cathepsin and tryptase in the mesenteric filament extract of *Cribrina artemisia* (DANA) digested gelatine solution most rapidly at pH 2.3 (citrate-HCl buffer), 4.8 (phosphate buffer) and 6.3 (phosphate buffer)-7.0~6.75 (glycocoll buffer)-7.3 (borax-boric acid buffer), respectively.

The pepsinase was found also in the gastric fluids in but minute traces. And tryptase was demonstrated also in the stomodaeum extract and unfiltered gastric fluid, and it was most active at pH 6.3 (phosphate buffer), 7.0~6.75 (glycocoll buffer) and 7.3 (borax-boric acid buffer) in the mesenteric filament extract. But those authors did not know the presence of a cathepsin: I have demonstrated the presence of cathepsin in the extracts of mesenteric filament and tentacle which is accelerated by the addition of cysteine hydrochloride solution for the digestion of gelatine at pH about 4.8.

ABDERHALDEN and HEISE (1909) reported the presence of a polypeptidase in *Actinia equina* by using glycyl-l-tyrosine as the substrate. ISHIDA (1936) confirmed the presence of a dipeptidase in the mesenteric filament extract and gastric fluid of *Actinia mesembryanthemum*, which digests glycyl-glycine at optimum pH about 8.29 (borax-HCl buffer). I

have been able to find amino-polypeptidase, carboxy-polypeptidase and dipeptidase, which digest dl-leucyl-glycyl-glycine, n-chloracetyl-l-tyrosine and glycyl-glycine respectively, in the glycerine extracts of mesenteric filament and stomodaeum of *Cribrina artemisia* (DANA); but carboxy-polypeptidase was absent in the stomodaeum extract. Amino-polypeptidase has an optimum pH 8.7 in the mesenteric filament extract and more active digestivity than in the stomodaeum extract. While, on the other hand, dipeptidase in the stomodaeum extract was more active than in the mesenteric filament extract and its optimum pH was 7.5. Carboxy-polypeptidase has an optimum pH 8.15 in the mesenteric filament extract. No one has yet investigated such polypeptidases in coelenterates.

BODANSKY (1923) found that the unfiltered gastric fluid of *Metridium marginatum* contains trypsin. But it is too weak to be investigated. He concluded that in *Metridium* the digestive enzymes are present mostly intracellularly. Partial digestion of food, however, may take place within the digestive cavity as a result of autolytic changes in food and to some extent by extracellular enzymes originating from the cell lining of the mesenteric filaments. According to ISHIDA (1936), pepsinase, tryptase and dipeptidase are in the mesenteric filaments of *Actinia mesembryanthemum*, whose glycerine extracts are entirely the same as those found in the gastro-vascular fluid because these enzymes found in the former have respectively the same optimum pH as those found in the latter, and then he concluded that the enzymes in the gastric fluid have their origin in the cells lining the mesenteric filament.

Although I have not enough ascertained it in *Cribrina artemisia* (DANA), my findings may support these conclusions as the pepsinase and tryptase in the gastric fluid was found in traces in fast.

#### SUMMARY

1. Proteolytic enzymes in *Cribrina artemisia* (DANA) have been investigated.

2. As proteinases (gelatine as the substrate), pepsinase, cathepsin, and tryptase are demonstrated and as peptidases, amino-polypeptidase (dl-leucyl-glycyl-glycine as the substrate), carboxy-polypeptidase (n-chloracetyl-l-tyrosine as the substrate) and dipeptidase (glycyl-glycine as the substrate) are detected.

3. Pepsinase is demonstrated in the mesenteric filament extract, unfiltered and filtered gastric fluid but it is found in traces in the both

gastric fluids. Optimum pH of pepsinase is 2.3 (citrate-HCl buffer) in the mesenteric filament extract.

4. Cathepsin is demonstrated in the extracts of mesenteric filament and tentacle, and its optimum pH is 4.8 (phosphate buffer) in the former. Moreover no one has yet investigated such enzyme in actinians.

5. Tryptase is detected in the extracts of mesenteric filament and stomodaeum and unfiltered gastric fluid, and its optimum pH is 6.3 (phosphate buffer), 7.0~6.75 (glycocoll buffer) and 7.3 (borax-boric acid buffer) in the mesenteric filament extract.

6. Amino-polypeptidase is demonstrated in the extracts of mesenteric filament and stomodaeum, and its optimum pH is 8.7 (glycocoll buffer) in the former.

7. Carboxy-polypeptidase is detected only in the mesenteric filament extract and its optimum pH is 8.15 (glycocoll buffer).

8. Dipeptidase is demonstrated in the extracts of mesenteric filament and stomodaeum, and its optimum pH is 7.5 (borax-boric acid buffer) in the latter.

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# ÜBER DAS REDOXPOTENTIAL DER SUSPENSION LEBENDER HEFEZELLEN

VON

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(Mit fünf Figuren)

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## 1. EINLEITUNG.

Schon im Jahre 1911 berichtete M. C. POTTER, dass eine Potentialdifferenz zwischen den Edelmetallelektroden, deren eine in die mit Hefen oder Bakterien geimpfte Kulturflüssigkeit, und deren andere in sterilisierte Flüssigkeit eingetaucht wurde, auftrat. Seitdem hat er fortwährend bis vor kurzem über dieses Problem mit der Ansicht, dass die bei der Zersetzung organischer Substraten entstehende elektrische Energie für die daran beteiligten Organismen von Nutzen sein muss, untersucht (1915, 1929, 1930, 1934 und 1935).

L. J. GILLESPIE (1920) mass ein Potential im lebende Mikroorganismen enthaltenden Kulturmedium mit Hilfe einer indifferenten Elektrode, und deutete es als erster als das Reduktionspotential. Danach sind zahlreiche Bestimmungen des Redoxpotentials in mikrobienhaltigen Medien gemacht worden und durch diese Arbeiten ist es klar geworden, dass ein Redoxpotential im genannten Medium mit dem Stoffwechselvorgang der Mikroorganismen in näherem Zusammenhang steht. Auch hat die Berücksichtigung des Redoxpotentials immer das grosse Verdienst gehabt, dazu zu leiten, in den zahlreichen mikrobiologischen Problemen eine bessere Einsicht zu geben.

In bezug auf das Redoxpotential in der Hefesuspension sind bis jetzt einige Beobachtungen gemacht worden. Um darüber eine Übersicht gewinnen zu können, werden hier die wenigen Arbeiten kurz erwähnt werden.

R. K. CANNAN, B. COHEN und W. M. CLARK (1926) führten einige grundlegende und wertvolle Untersuchungen über das Redoxpotential der Hefesuspension aus. Aber hier muss darauf hingewiesen werden, dass sie nur das in wässriger Suspension der Trockenhefe auftretende Redoxpotential, dessen Beziehung zur Gärung schwierig zu übersehen ist, massen.



E. AUBEL, E. AUBERTIN und L. GENEVOIS (1929) bestimmten unter der anaeroben Bedingung das Redoxpotential in glukosehaltigen Kulturmedien der Hefezellen mit Hilfe der Redoxindikatoren und fanden dabei, dass bei  $\text{pH}=7,2$  der festgestellte Endwert des Potentials im Medium zwischen  $-160$  und  $-200$  mV. lag. Seitdem wurden die Redoxpotentiale in der glukosehaltigen, auf  $\text{pH}=5,4$  gepufferten Hefesuspension von A. J. KLUYVER und J. C. HOOGERHEIDE (1934) potentiometrisch gemessen und auch der Stoffwechseltypus der betreffenden Zellen gleichzeitig festgestellt. Dabei fanden sie, dass ein Potentialniveau für verschiedene Hefearten zwischen  $+80$  und  $+100$  mV. lag, und dieses Niveau von den verwendeten Hefearten, von den benutzten Zuckerarten und von der Zuckerkonzentration unabhängig war. Es lässt sich sogleich entnehmen, dass dieses Ergebnis sich wesentlich von dem früher von E. AUBEL, E. AUBERTIN und L. GENEVOIS erhaltenen unterscheidet.

Ganz kürzlich wiesen C. FROMAGEOT und P. DESNUELLE (1936) auf diesen Punkt hin und mit der Ansicht, dass die wesentliche Verschiedenheit der Redoxpotentiale in der Suspension lebender Hefezellen mit der Fähigkeit der Hefe zusammenhängt, die Synthese des Alanins zu verwirklichen, bestimmten sie die Redoxpotentiale in der zuckerhaltigen, auf  $\text{pH}=6,4$  gepufferten Hefesuspension mit Hilfe der Redoxindikatoren. Dabei ergab sich unter den genannten Bedingungen das Redoxpotential von  $-144$  bis  $-184$  mV.

Dank der obengenannten Arbeiten liegt uns als Tatsache bewiesen vor, dass die Endwerte der Redoxpotentiale in den Hefesuspensionen je nach der Natur der messenden Methode (der potentiometrischen Messung oder der Redoxindikatorenmessung) wesentlich voneinander abweichen können. Nun entsteht die Frage, inwieweit betrifft des Redoxpotentials die potentiometrische Messung mit der Indikatorenmessung übereinstimmen soll.

Um diese Frage klarzustellen, habe ich die Versuche über das Redoxpotential in der Suspension lebender Hefezellen mit Hilfe der potentiometrischen Messung beabsichtigt.

## 2. METHODIK.

Die Versuche wurden folgenderweise ausgeführt: Die Versuchsanordnung wurde nach A. J. KLUYVER und J. C. HOOGERHEIDE (1934) bereitgestellt. Das Redoxpotential und das eine Stoffwechselprodukt, d. h. die Kohlensäure, der betreffenden Hefesuspension wurden dadurch gleichzeitig bestimmt, dass das Gärungsgefäß des WARBURG'schen Apparates mit einer

Platinelektrode verbunden wurde.

An diesem Gärungsgefäß wurde eine kleine Verbesserung folgendermassen gemacht. Das Gefäß wurde ausser einer blanken Platinelektrode und einem KCl-Agarheber mit einem Glasrohr, durch dessen Hahn der Stickstoff, im Gegensatz zur gewöhnlichen Erfahrung, um die darin befindliche Luft zu vertreiben, in das Gefäß eingeleitet wurde, so ausgerüstet, dass das eine Ende dieses Glasrohrs möglichst dem Boden des Gefässes näher kam. Damit gelang es mir, bei der Ersetzung der im Gefäß befindlichen Luft durch den Stickstoff das Eindringen der Luft vollständig zu vermeiden.

Die Reinigung des Stickstoffs wurde vorsichtig so ausgeführt, dass dieses Gas von der Bombe aus über geglühte Kupfernetze, welche vorher vollständig reduziert waren, ziemlich langsam übergeleitet und dann, um das erhitzte Gas zu kühlen, durch die alkalische Lösung des Natriumhydrosulfits geführt wurde. Zur Vermeidung der Eindifundierung des Sauerstoffs in der Luft durch die Leitungsröhre wurden Bleiröhre für die Leitung des Stickstoffs angewandt, mit Ausnahme von der Verbindungsstelle, wo dicke Gummiröhren benutzt wurden.

Die in diesen Versuchen als Suspension angewandten Unterhefen stammten aus der Kirin-Bierbrauerei in Sendai, Japan, und wurden in der zuckerhaltigen mineralischen Flüssigkeit rein kultiviert. Die Suspension wurde folgendermassen bereitet. Der glukosehaltige (3%), auf  $\text{pH}=5,4$  gepufferte Phosphatpuffer wurde bis auf 0,4% mit den zentrifugierten Hefen suspendiert, welche vorher mit dem genannten Puffer zweimal gewaschen wurden. Von dieser Hefesuspension wurden dann 5 ccm in das Gärungsgefäß einpipettiert.

Zunächst wurde das Gärungsgefäß mit dem Manometer verbunden und im Wasserthermostat ( $30^{\circ}\text{C}$ ) aufgestellt; und sogleich danach das Potential mit Hilfe der blanken Platinelektrode gegenüber der  $\text{N}10^{-1}\text{-KCl}$ -Kalomelektrode bestimmt. Nach einer Stunde von dieser Zeit wurde der Stickstoff, der vollständig vom beigemengten Sauerstoff befreit war, durch das Gaszufuhrrohr während  $2\frac{1}{2}$  oder 3 Stunden hindurch in das Gefäß eingeleitet, um die vollkommene Ersetzung der im Gefäß befindlichen Luft zu verwirklichen. Nach der Unterbrechung der Stickstoffzufuhr wurde jede Halbstunde die entwickelte Kohlensäuremenge manometrisch gemessen.

Zur Messung des Potentials wurde die Kompensationsmethode benutzt und der Messapparat wurde mit einem Kapillarelektrometer als Nullinstrument und einem Messbrückenpotentiometer ausgerüstet.

Die im folgenden wiedergegebenen Potentialwerte sind alle auf die Normalwasserstoffelektrode bezogen angegeben.

### 3. DAS REDOXPOTENTIAL DER HEFESUSPENSION UNTER STRENG ANAEROBER BEDINGUNG.

Um den Endwert des Redoxpotentials der Hefesuspension während des Gärungsvorganges zu untersuchen, muss die streng anaerobe Bedingung vorausgesetzt werden, weil unter aerober Bedingung nicht nur die Atmung neben der Gärung vor sich geht, sondern auch der Sauerstoff direkten Einfluss auf das betreffende Redoxsystem ausübt, sodass die Verhältnisse sehr kompliziert ausfallen müssen. In der anaeroben Bedingung dagegen kann man einfach, ohne Rücksicht auf den Einfluss des Sauerstoffs, das Redoxpotential der Hefesuspension behandeln. Aus dieser Überlegung wurde zunächst der Versuch unter streng anaerober Bedingung gemacht.

Die aus diesem Versuch erhaltenen Daten sind in Tabelle 1 zusammengestellt und in Abb. 1 graphisch wiedergegeben.

TABELLE 1.

*Redoxpotential und Gärung der Suspension von Saccharomyces cerevisiae in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, in Stickstoff. Bei \* wurde Stickstoff eingeleitet, bei \*\* wurde Stickstoffzufuhr unterbrochen.*

Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt	Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt
0	—	+0,4403	270	103,7	-0,1810
15	—	+0,3988	300	129,4	-0,1810
30	—	+0,3988	330	134,2	-0,1810
60*	—	+0,3980	360	135,9	-0,1803
90	—	+0,2863	390	137,4	-0,1787
105	—	+0,1300	420	134,2	-0,1779
120	—	-0,0276	450	138,2	-0,1779
135	—	-0,1123	480	147,1	-0,1779
150	—	-0,1646	510	138,2	-0,1756
165	—	-0,1795	540	144,7	-0,1740
180	—	-0,1795	570	145,5	-0,1732
195	—	-0,1795	600	141,4	-0,1732
210**	—	-0,1795	630	154,3	-0,1732
240	102,1	-0,1810			

Es lässt sich nun entnehmen, dass das Potential in Luft erheblich höher ausgefallen ist und gleich nach der Durchleitung des Stickstoffs in das Gefäß momentane Potentialsenkung auftritt. Nach etwa 2 Stunden stellt das Potential sich auf einem definitiven Niveau ein, worauf es danach lange Zeit annähernd konstant bleibt, obwohl die Stickstoffzufuhr unterbrochen wird. Durch die Unterbrechung der Stickstoffzufuhr wird dabei

keine Veränderung des Potentialverlaufs hervorgerufen. Dies ist zweifellos bemerkenswert, weil das Potentialniveau durch die im Gefäß noch befindliche Sauerstoffspur verändert werden sollte, wenn der angewandte Stickstoff nicht vollständig von dem Sauerstoff befreit wäre.

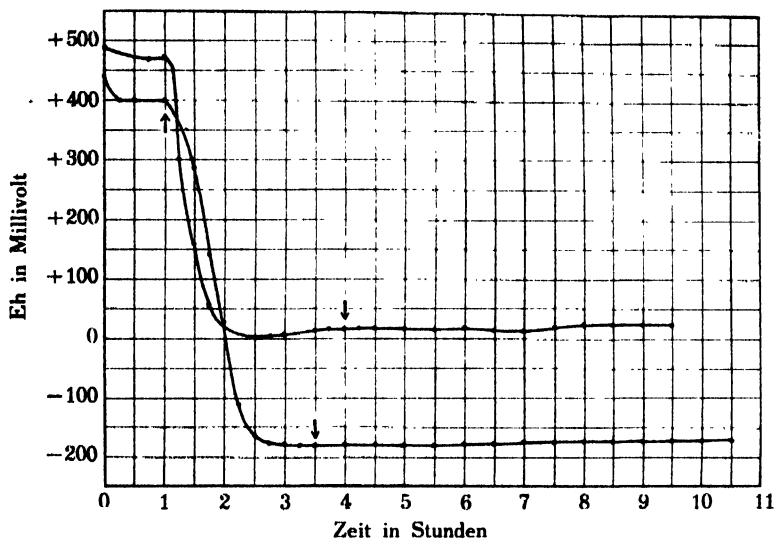


Abb. 1. Redoxpotentiale der Suspension von *Saccharomyces cerevisiae*. Obere Linie im ungereinigten Stickstoff, untere Linie im reinen Stickstoff. Bei  $\uparrow$  wurde Stickstoff eingeleitet, bei  $\downarrow$  wurde Stickstoffzufuhr unterbrochen.

Es geht aus diesem Ergebnis hervor, dass der Endwert des Redoxpotentials in der Hefesuspension unter der genannten anaeroben Bedingung bei pH=5,4 etwa  $-180$  mV. ist. Dieser Wert ist im Vergleich zum Befunde A. J. KLUYVERS und J. C. HOGERHEIDES wesentlich niedriger.

Die dabei durch Gärung entwickelten Kohlensäuremengen sind als  $Q_{CO_2}^{N_2}$  gezeichnet in der Tabelle 1 und in Abb. 4 graphisch wiedergegeben. Diese Werte sind verhältnismässig niedrig. Dies ist wahrscheinlich darauf zurückzuführen, dass die manometrischen Apparate wegen des Gewichts ihrer Anhänge (der Elektrode, des KCl-Agarhebers und des Gaszufuhrrohrs) auf die Schüttelung verzichtet wurden. Es zeigt sich jedoch, dass die Kohlensäuremenge anfangs relativ klein ist, dann aber annähernd konstant bleibt.

Dass die eingestellte Redoxpotentialzeitkurve mit der Gärkurve beinahe parallel geht, scheint mir von Bedeutung. Dies spricht dafür, dass in diesem Falle die definitive Einstellung des Redoxpotentials nur durch das Gleichgewicht des ganzen Systems hervorgerufen wird. Das wahre Gleich-

gewicht des Systems kann bekanntlich nur erst unter streng anaerober Bedingung erreicht werden. Befindet sich eine Spur des Sauerstoffs im Gefäß, so werden die Verhältnisse sehr kompliziert, sodass in bezug auf die Gärung, wie oben erwähnt, kein wahres Gleichgewicht stattfinden kann und folglich das damit in näherem Zusammenhang stehende Redoxpotential beträchtlich beeinflusst wird.

Diese Berücksichtigung zwingt mich zu prüfen, wie das Potential der Hefesuspension durch die Zufuhr des ungereinigten Stickstoffs verändert wird.

#### 4. DAS REDOXPOTENTIAL DER HEFESUSPENSION IM UNGEREINIGTEN STICKSTOFF.

Der Stickstoff wurde direkt aus der Bombe in das Gärungsgefäß ohne Überleitung über geglühtes Kupfer eingeleitet. Sonst war die Anordnung ganz gleich wie beim vorigen Versuch.

Die Ergebnisse sind in Tabelle 2 zusammengestellt und in Abb. 1 graphisch wiedergegeben.

TABELLE 2.

*Redoxpotential der Suspension von Saccharomyces cerevisiae in 3%iger Glukoselösung, Phosphatpuffer,  $pH=5,4$ , in ungereinigtem Stickstoff. Bei \* wurde Stickstoff eingeleitet, bei \*\* wurde Stickstoffzufuhr unterbrochen.*

Zeit in Min.	Eh in Millivolt	Zeit in Min.	Eh in Millivolt
0	+0,4871	270	+0,0173
45	+0,4691	300	+0,0165
60*	+0,4691	330	+0,0222
75	+0,2970	360	+0,0187
90	+0,1542	390	+0,0165
105	+0,0673	420	+0,0165
165	+0,0083	450	+0,0247
180	+0,0083	480	+0,0247
210	+0,0116	510	+0,0255
225	+0,0116	540	+0,0247
240**	+0,0116	570	+0,0247
255	+0,0173		

Wie an dieser Tabelle ersichtlich ist, verläuft anfangs die Potentialzeitkurve ähnlich wie die im vorigen Versuch, alsdann stellt sie sich um etwa +20 mV. ein, wo sie ziemlich konstant ohne weiteres Absinken bleibt. Dieses Potentialniveau ist, wie zu erwarten war, bedeutend höher als das im vorigen Versuch. Diese Erkenntnis ist von grosser Wichtigkeit, weil selbst eine Spur des Sauerstoffs, welche im ungereinigten Stickstoff vermischt vorhanden wäre, einen schweren Einfluss auf das Redoxpotential

der Hefesuspension ausüben müsste.

Ja es muss hier mit Recht betont werden, dass um das Redoxpotential der Hefesuspension in vollständigem Gleichgewicht des ganzen Systems zu bestimmen, die streng anaerobe Bedingung unentbehrlich ist.

Folglich muss es nun in Betracht bezogen werden, ob im vorigen Versuch unter dem Abschnitt 3 die im Gefäss befindliche Luft durch den reinen Stickstoff vollständig ersetzt werden konnte oder nicht. Um darüber Klarheit zu gewinnen, wird ein Versuch wie folgt beabsichtigt.

### 5. DAS REDOXPOTENTIAL DER HEFESUSPENSION IN GEGENWART VON WASSERSTOFF ODER VON STICKSTOFF.

Die im Gefäss befindliche Luft wurde zuerst durch den Wasserstoff, welcher durch Überleitung über geglühte Kupfernetze von dem beigemengten Sauerstoff befreit worden war, vertrieben und dann durch den obengenannten reinen Stickstoff ersetzt. Übrige Anordnung war ganz gleich wie bei vorhergehenden Versuchen.

Die dabei erhaltenen Ergebnisse sind in Tabelle 3 zusammengestellt und in Abb. 2 graphisch wiedergegeben.

TABELLE 3.

*Redoxpotential der Suspension von Saccharomyces cerevisiae in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, zuerst Wasserstoff und dann Stickstoff eingeleitet.*

Zeit in Min.	Eh in Millivolt	Bemerkung	Zeit in Min.	Eh in Millivolt	Bemerkung
0	+0,5080		210	-0,3022	- N <sub>2</sub> einge- leitet
60	+0,4644	- H <sub>2</sub> einge- leitet	215	-0,2785	
75	+0,2307		225	-0,2531	
77	+0,1880	- H <sub>2</sub> -Zufuhr unter- brochen	240	-0,1785	- N <sub>2</sub> -Zufuhr unter- brochen
82	+0,0448		270	-0,1721	
92	-0,2767		300	-0,1721	
105	-0,3113		330	-0,1703	
120	-0,3113		360	-0,1703	
150	-0,3113		390	-0,1703	
180	-0,3022		420	-0,1703	

Aus diesen Ergebnissen geht hervor, dass gleich nach der Durchleitung des Wasserstoffs in das Gefäss eine erhebliche Potentialsenkung auftritt und schliesslich das Potential ein wesentlich negatives Niveau erreicht, worauf es ziemlich konstant bleibt. Durch die Unterbrechung der Wasserstoffzufuhr wird keine plötzliche Änderung des Potentialverlaufs hervorgerufen. Wird aber der sehr reine Stickstoff in das Gefäss geführt, um den Wasserstoff zu vertreiben, so springt das Potential sofort auf etwa

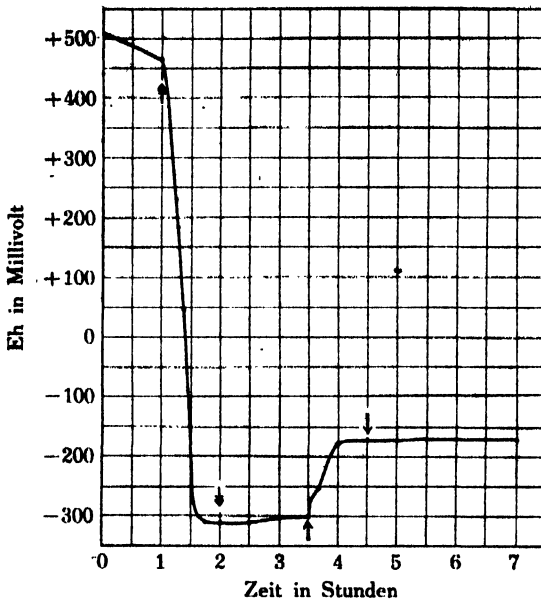


Abb. 2. Redoxpotential der Suspension von *Saccharomyces cerevisiae* in 3%iger Glukoselösung, zuerst Wasserstoff und dann Stickstoff eingeleitet. Bei ↑ Wasserstoff eingeleitet, bei ↓ Wasserstoffzufuhr unterbrochen, bei ↑ Stickstoff eingeleitet, bei ↓ Stickstoffzufuhr unterbrochen.

stoff vorhanden wäre, der wahre Endwert des Potentials in der betreffenden Hefesuspension unter der anaeroben Bedingungen niedriger als der dabei gefundene Potentialwert stehen sollte. Wie aus Tabelle 3 und Abb. 2 ersichtlich ist, steht aber das beobachtete Potentialniveau in reinem Stickstoff durchaus in Übereinstimmung mit demselben, welches in Abschnitt 3 festgestellt war. Diese Tatsache ist ein guter Beweis dafür, dass bei dem in Abschnitt 3 erwähnten Versuche keine Spur des Sauerstoffs vorhanden war, sodass der dabei erhaltene Endwert des Redoxpotentials als der wahre der betreffenden Hefesuspension unter streng anaerober Bedingung anerkannt werden kann.

#### 6. DAS REDOXPOTENTIAL DER HEFESUSPENSION IN GEGENWART VON REDOXINDIKATOREN.

Es schien mir sehr wünschenswert, den Potentialverlauf bei der Hefesuspension unter Zusetzung irgendeines geeigneten Redoxindikators zu beobachten. Denn es war in der Literatur manchmal gesagt, dass durch

—170 mV. und bleibt hier lange Zeit konstant.

In diesem Falle ist es sehr wahrscheinlich, dass das gefundene Potential in Gegenwart von Wasserstoff, etwa —310 mV., gerade dem Potentialwert der Wasserstoffelektrode für pH=5,4 entspricht, was durch die eventuelle Funktion der angewandten Platinelektrode wie eine Wasserstoffelektrode verursacht werden könnte.

Es ist nun bedenklich, dass wenn eine Spur des Sauerstoffs infolge der unvollkommenen Ersetzung der Luft durch den Stick-

Zugabe des geeigneten Redoxindikators die Einstellungsgeschwindigkeit erhöht werden konnte, oder dass erst in Gegenwart von Redoxindikatoren ein charakteristisches Potential bestimmt werden konnte. Meine oben erwähnten Versuche haben aber sichergestellt, dass es nicht immer der Fall sein kann, und dass sogar ohne Zusatz von Redoxindikatoren ziemlich stabile und mehr oder weniger empfindliche Potentialverläufe beobachtet werden können.

So entsteht nun eine Frage, inwieweit der zeitliche Verlauf des Elektrodenpotentials durch die Gegenwart von Redoxindikatoren beeinflusst wird. Der diesbezügliche Versuch wurde folgendermassen ausgeführt: Als Redoxindikatoren wurden vier Farbstoffe, nämlich Methylenblau, Indigotetrasulfonat, Indigotrisulfonat und Janusgrün, benutzt. Die Versuchsanordnung war hier auch dieselbe wie beim Versuch im Abschnitt 3 vorgenommen war, ausgenommen der Zugabe einer Spur der genannten Redoxindikatoren in die betreffende Hefesuspension in einem Grad, bei dem die betreffende Farbe erst erkannt werden konnte.

Die erhaltenen Ergebnisse sind in Tabellen 4-7 zusammengestellt und in Abb. 3 graphisch wiedergegeben.

TABELLE 4.

*Redoxpotential und Gärung der Suspension von Saccharomyces cerevisiae in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, in Stickstoff. Methylenblau zugesetzt. Bei \* Stickstoff eingeleitet, bei \*\* Stickstoffzufuhr unterbrochen.*

Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt	Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt
0	—	+0,4942	345	—	-0,1898
30	—	+0,4742	360	118,3	-0,1898
45	—	+0,4410	375	—	-0,1882
60*	—	+0,4394	390	128,9	-0,1857
75	—	+0,4360	405	—	-0,1857
90	—	+0,4034	420	121,3	-0,1857
105	—	+0,2995	435	—	-0,1849
120	—	+0,2250	450	122,8	-0,1849
135	—	+0,1808	465	—	-0,1849
150	—	+0,1195	480	129,7	-0,1832
165	—	+0,0925	495	—	-0,1832
180	—	+0,0867	510	137,3	-0,1824
195	—	+0,0557	525	—	-0,1824
210	—	-0,0253	540	132,0	-0,1800
225	—	-0,0932	555	—	-0,1800
240**	—	-0,1423	570	139,6	-0,1800
255	—	-0,1783	585	—	-0,1792
270	93,9	-0,1882	600	133,3	-0,1792
285	—	-0,1898	615	—	-0,1792
300	104,5	-0,1898	630	144,2	-0,1792
315	—	-0,1898	645	—	-0,1783
330	103,8	-0,1898	660	134,4	-0,1783



TABELLE 5.

Redoxpotential und Gärung der Suspension von *Saccharomyces cerevisiae* in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, in Stickstoff. Indigotetrasulfonat zugesetzt. Bei \* Stickstoff eingeleitet, bei \*\* Stickstoffzufuhr unterbrochen.

Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt	Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt
0	—	+0,4755	360	118,1	-0,1713
60*	—	+0,4646	390	123,8	-0,1713
90	—	+0,4122	405	—	-0,1730
105	—	+0,3721	420	128,6	-0,1746
120	—	+0,3196	435	—	-0,1746
135	—	+0,2188	450	132,6	-0,1755
150	—	+0,1254	480	127,0	-0,1763
165	—	+0,0679	495	—	-0,1763
180	—	+0,0321	510	129,4	-0,1780
195	—	+0,0287	525	—	-0,1805
225	—	+0,0246	540	130,2	-0,1788
240**	—	+0,0004	555	—	-0,1788
255	—	-0,0521	570	135,9	-0,1788
270	91,6	-0,1080	585	—	-0,1777
285	—	-0,1363	600	143,9	-0,1772
300	105,3	-0,1571	615	—	-0,1772
315	—	-0,1638	630	140,7	-0,1772
330	109,3	-0,1713	645	—	-0,1772
345	—	-0,1713	660	147,0	-0,1772

TABELLE 6.

Redoxpotential und Gärung der Suspension von *Saccharomyces cerevisiae* in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, in Stickstoff. Indigotrisulfonat zugesetzt. Bei \* Stickstoff eingeleitet, bei \*\* Stickstoffzufuhr unterbrochen.

Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt	Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt
0	—	+0,4938	360	121,3	-0,0997
60*	—	+0,4529	390	125,2	-0,1172
90	—	+0,3921	405	—	-0,1230
105	—	+0,3330	420	122,1	-0,1296
120	—	+0,2280	435	—	-0,1360
135	—	+0,1338	450	131,3	-0,1488
150	—	+0,0771	480	134,3	-0,1686
165	—	+0,0504	495	—	-0,1686
180	—	+0,0179	510	129,7	-0,1721
195	—	+0,0062	525	—	-0,1713
225	—	-0,0104	540	120,5	-0,1730
240**	—	-0,0171	555	—	-0,1721
255	—	-0,0188	570	135,0	-0,1721
270	103,0	-0,0188	585	—	-0,1686
285	—	-0,0188	600	127,4	-0,1705
300	108,0	-0,0413	615	—	-0,1680
315	—	-0,0571	630	129,0	-0,1680
330	131,0	-0,0771	645	—	-0,1680
345	—	-0,0871	660	144,2	-0,1680

TABELLE 7.

Redoxpotential und Gurung der Suspension von *Saccharomyces cerevisiae* in 3%iger Glukoselosung, Phosphatpuffer, pH=5,4, in Stickstoff. Janusgrun zugesetzt. Bei \* Stickstoff eingeleitet, bei \*\* Stickstoffzufuhr unterbrochen.

Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt	Zeit in Min.	$Q_{CO_2}^{N_2}$	Eh in Millivolt
0	—	+0,4631	345	—	-0,1890
30	—	+0,4508	360	127,0	-0,1890
45	—	+0,4058	375	—	-0,1882
60*	—	+0,3886	390	131,0	-0,1873
75	—	+0,3486	405	—	-0,1873
90	—	+0,3126	420	126,8	-0,1865
105	—	+0,1375	435	—	-0,1865
120	—	+0,0188	450	125,4	-0,1865
135	—	-0,0785	465	—	-0,1865
150	—	-0,1063	480	129,4	-0,1857
165	—	-0,1726	495	—	-0,1850
180	—	-0,1808	510	131,8	-0,1850
195	—	-0,1890	525	—	-0,1841
210	—	-0,1890	540	141,5	-0,1841
225	—	-0,1890	555	—	-0,1841
240**	—	-0,1890	570	126,1	-0,1832
255	—	-0,1890	585	—	-0,1832
270	102,9	-0,1890	600	134,2	-0,1832
285	—	-0,1898	615	—	-0,1832
300	110,1	-0,1898	630	127,0	-0,1824
315	—	-0,1898	645	—	-0,1824
330	111,0	-0,1898	660	138,7	-0,1824

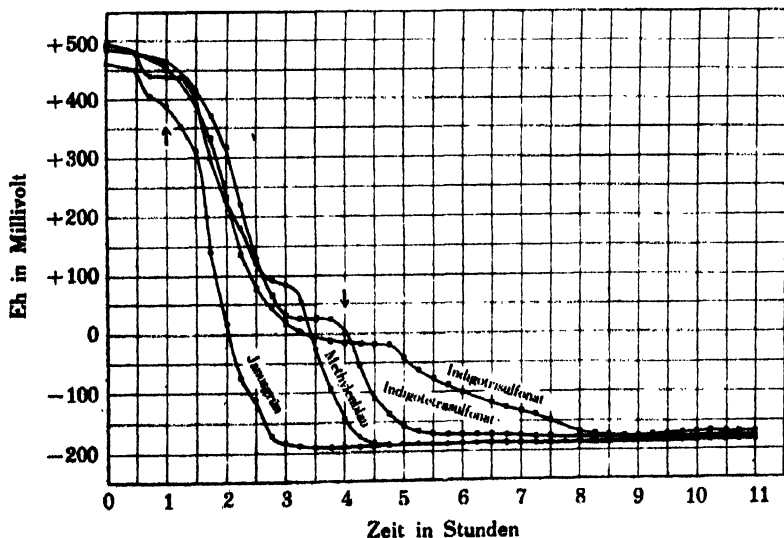


Abb. 3. Redoxpotentiale der Suspension von *Saccharomyces cerevisiae*. Methylblau, Indigotetrakisulfonat, Indigotrisulfonat oder Janusgrun zugesetzt. Bei ↑ Stickstoff eingeleitet, bei ↓ Stickstoffzufuhr unterbrochen.

Wie aus diesen Ergebnissen ersichtlich ist, zeigt das Potential der Hefesuspension bei der Zugabe jedes Redoxindikators einen besonderen charakteristischen Verlauf. Und zwar beginnen in Gegenwart dieser Indikatoren die Potentialzeitkurven in allen Fällen zur Zeit der Stickstoffzufuhr ähnlich abzusinken, wie beim Versuch ohne Indikatoren, trotzdem flachen sie unterwegs, bevor sie die Endwerte der Potentiale erreichen, um besonderen Stellen für die betreffenden Redoxindikatoren, ab.

Es ist aber bemerkenswert, dass alle Potentiale unabhängig von den zugesetzten Redoxindikatoren schliesslich dasselbe Niveau, etwa von  $-170$  bis  $-180$  mV., erreichen können, und darauf lange Zeit ziemlich konstant bleiben. Dieses Niveau stimmt mit dem ohne Indikatoren festgestellten annähernd überein.

Was nun die Abflachung der Potentialzeitkurven anbelangt, so liegt sie für Methylenblau um etwa  $+80$  mV., für Indigotetrasulfonat um etwa  $+30$  mV. und für Indigotrisulfonat um etwa  $-15$  mV. Aber beim Janusgrün wird keine Abflachung der Potentialzeitkurve beobachtet. Auf diese letzte Erscheinung werden wir später wieder zurückkommen.

Es ist bemerkenswert, dass diese Potentialwerte, worum sie abflachen, für die ersten drei Redoxindikatoren gerade den betreffenden Normalpotentialwerten bei  $\text{pH}=5,4$  entsprechen, d. h.  $+77$  mV. für Methylenblau,  $+41$  mV. für Indigotetrasulfonat und  $+8$  mV. für Indigotrisulfonat (nach W. M. CLARK). Aus diesen Tatsachen wäre es berechtigt anzunehmen, dass die in obengenannten Versuchen beobachteten Abflachungen der Potentialzeitkurven sicher auf die Verzögerungen der Potentialabfälle durch die beschwerenden Wirkungen der zugesetzten Redoxindikatoren zurückzuführen sind.

Und zwar bekräftigte diese Annahme weiter die Tatsache, dass die vollkommenen Entfärbungen dieser Redoxindikatoren, welche durch ihre vollständigen Reduktionen verursacht werden, gleich nach den Wiederabfällen der Potentialzeitkurven auftreten.

Wie schon R. K. CANNAN, B. COHEN und W. M. CLARK (1926) beobachteten, überschreitet das Potential des betreffenden Systems im allgemeinen von Fall zu Fall die speziellen Bereiche der verschiedenen Redoxindikatoren; und wenn ein gewisser Redoxindikator in die Hefesuspension zugesetzt wird, wird die Abfallgeschwindigkeit des Potentials in dem dem betreffenden Normalpotential entsprechenden Bereiche sehr langsam, wobei gleichzeitig die Farbe dementsprechend allmählich abblasst, und erst nach völliger Entfärbung setzt der Potentialabfall sich wieder, wie früher, bis auf den Endwert fort. Es ist selbstverständlich, dass je

niedriger das Normalpotential des Indikators liegt, desto länger es zur Entfärbung, d. h. zur völligen Reduktion durch das andere System, dauert. Aus meinen Versuchen geht auch hervor, dass die Zeit zur Entfärbung der ersten drei Redoxindikatoren bei Methylenblau am kürzesten und bei Indigotrisulfonat am längsten ist.

Es muss nun die Frage beantwortet werden, warum bei dem Versuch mit Janusgrün vor der Erreichung des Endwertes des Potentials gar keine Abflachung der Potentialzeitkurve auftritt. Darüber sei nur folgendes gesagt. Bekanntlich hängt die Frage, in welchen Massen die Verzögerung des Potentialabfalls durch die beschwerende Wirkung des zugesetzten Redoxindikators beeinflusst wird, sowohl von dem Intensitätsfaktor (dem Potential) als auch von dem Kapazitätsfaktor (der reduziert vorliegenden Farbstoffmenge) ab. Wie oben erwähnt, findet sich stets die beschwerende Wirkung des Redoxindikators im Bereich seines Normalpotentials für gegebenes pH, soweit meine vorliegende Versuche reichen. Da das Normalpotential von Janusgrün für  $\text{pH}=5,4$   $-140$  mV. (nach W. M. CLARK) ist, so ist es zu ersehen, dass dieses Normalpotential dem Endwert des Potentials betreffender Hefesuspension sehr nahe steht. Deshalb ist lange Zeit zur vollständigen Reduktion erforderlich. Da Janusgrün infolge der Annäherung der Potentiale beider betreffenden Systeme dabei nicht vollständig reduziert werden konnte, obwohl teilweise Reduktion auftreten kann, wie eine schwache Abblassung daran deutet, so konnte der Wiederabfall des Potentials ebenfalls gar nicht beobachtet werden.

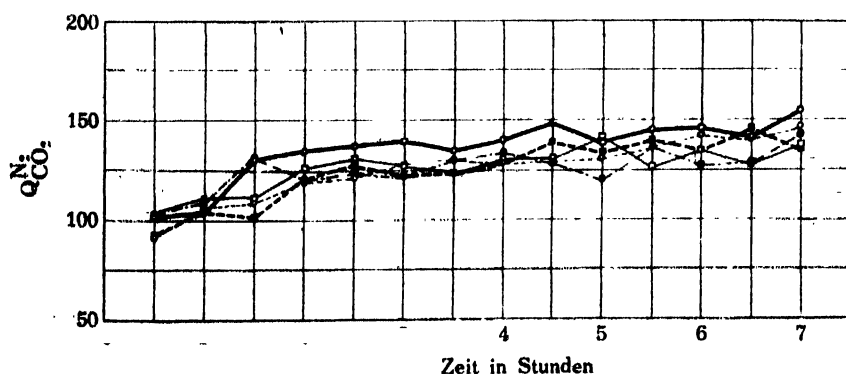


Abb. 4. Gärkurven. Zur Zeit 0 wurde die Stickstoffzufuhr in jedem Fall unterbrochen.

- |                                   |                                 |
|-----------------------------------|---------------------------------|
| ○ — — — ○ ohne Redoxindikator     | ■ — — — ■ mit Methylenblau      |
| ○ — — — ○ mit Indigotetrasulfonat | ● — — — ● mit Indigotrisulfonat |
| □ — — — □ mit Janusgrün           |                                 |

Mit diesen Versuchen ist also der Beweis gegeben, dass das Potential, welches in der Suspension lebender Hefezellen unter der streng anaeroben Bedingung bei  $\text{pH}=5,4$  festgestellt wird, nicht nur niedriger als jedes Normalpotential für Methylenblau, Indigotetrasulfonat, Indigotrisulfonat und Janusgrün, sondern auch demselben des letzteren sehr nahe sein muss.

Die dabei erhaltenen  $Q_{\text{CO}_2}^{\text{N}_2}$ -Werte sind ebenfalls in Abb. 4 graphisch wiedergegeben.

Man sieht daraus, dass diese Werte etwas niedriger als diejenigen beim Versuche ohne Zusatz von Redoxindikatoren sind, trotzdem die Gärkurven beinahe ähnlich verlaufen. Auch in diesen Fällen gehen die Gärkurven beinahe parallel mit den sich einstellenden Potentialzeitkurven nebeneinander.

#### 7. DAS REDOXPOTENTIAL DER HEFESUSPENSION IN GEGENWART VON METHYLENBLAU IN DEN VERSCHIEDENEN KONZENTRATIONEN.

Im vorigen Abschnitt sind einige Versuche beschrieben worden, in denen durch die charakteristischen beschwerenden Wirkungen, welche durch den Intensitätsfaktor und den Kapazitätsfaktor bedingt wurden, der Potentialverlauf ausserordentlich beeinflusst wurde.

Was nun den Einfluss des Intensitätsfaktors auf den Potentialverlauf betrifft, so wurden einige Versuche mit Redoxindikatoren, welche verschiedene Potentialbereiche hatten, vorgenommen. Die dabei erhaltenen Ergebnisse gaben uns einen Einblick, dass je näher der Potentialbereich eines Redoxindikators dem Endwert des Potentials der betreffenden Hefesuspension stand, desto stärker die Verzögerung des Potentialabfalls auftrat.

Es ist nun wünschenswert zu untersuchen, inwieweit die beschwerende Wirkung auf die Verzögerung des Potentialabfalls durch die Verschiedenheit der Kapazität desselben Redoxindikators verändert wird. Als der geeignete Redoxindikator wurde Methylenblau gewählt. Methylenblau wurde in das eine Gefäss 0,0001% und in das andere 0,0005% zugesetzt, nämlich war die Kapazität des letzteren fünfmal so gross wie diejenige des ersteren. Die Redoxpotentiale wurden in beiden gleichzeitig gemessen.

Die erhaltenen Ergebnisse sind in Tabelle 8 zusammengestellt und in Abb. 5 graphisch wiedergegeben.

TABELLE 8.

Redoxpotential der Suspension von *Saccharomyces cerevisiae* in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, in Stickstoff. Zugabe von Methylenblau in verschiedenen Konzentrationen. Bei \* Stickstoff eingeleitet, bei \*\* Stickstoffzufuhr unterbrochen.

Zeit in Min.	Methylenblau 0,0001%	Methylenblau 0,0005%	Zeit in Min.	Methylenblau 0,0001%	Methylenblau 0,0005%
	Eh in Millivolt	Eh in Millivolt		Eh in Millivolt	Eh in Millivolt
0	+0,5046	+0,4632	180**	+0,1316	+0,1378
30	+0,4632	+0,4553	195	+0,0567	+0,1263
45	+0,4579	+0,4667	210	-0,1056	+0,1193
60*	+0,4639	+0,4664	255	-0,1665	+0,0937
75	+0,4050	+0,3980	285	-0,1700	+0,0461
90	+0,3680	+0,3785	315	-0,1682	-0,1056
120	+0,3106	+0,2907	345	-0,1691	-0,1779
135	+0,2867	+0,2498	375	-0,1691	-0,1779
150	+0,1475	+0,1695	405	-0,1691	-0,1779
165	+0,1325	+0,1378			

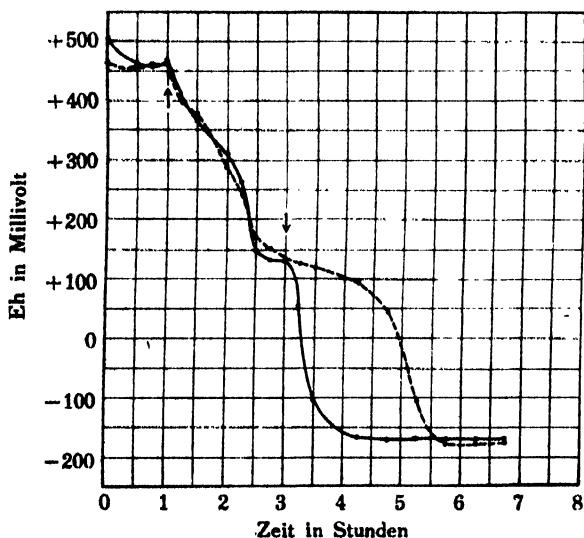


Abb. 5. Redoxpotentiale der Suspension von *Saccharomyces cerevisiae* in 3%iger Glukoselösung, Phosphatpuffer, pH=5,4, in Stickstoff. Bei ↑ Stickstoff eingeleitet, bei ↓ Stickstoffzufuhr unterbrochen.

○—○ 1.10<sup>-4</sup>%ige Methylenblauzugabe  
 ..... 5.10<sup>-4</sup>%ige Methylenblauzugabe

Aus Tabelle 8 und Abb. 5 geht hervor, dass die beiden Potentialverläufe anfangs ähnlich absinken und die Abflachungen der beiden Potentialzeitkurven zur gleichen Zeit auftreten, während die Potentiale das Bereich etwa +130mV. durchlaufen. Bei 0,0001%iger Methylenblauzugabe

schreitet das Potential in der Negativität wieder nach einiger Zeit fort, um den im vorigen Versuche gefundenen Endwert des Potentials zu erreichen. Bei 0,0005%iger Methylenblaugabe wird dagegen der Potentialabfall länger verzögert, obwohl der Endwert des Potentials trotzdem gleich wie beim vorigen Falle bleibt.

Von auffallender Bedeutung ist die Tatsache, dass die Potentiale in Gegenwart von Methylenblau, unabhängig von der Zeit ihrer Abfallsverzögerung, auf demselben Niveau einzustellen kommen. Obwohl die Abflachungen der Potentialzeitkurven höher als die theoretisch zu erwartenden lagen, dürfen die Ergebnisse dieser Versuche ohne weiteres dafür sprechen, dass durch die beschwerende Wirkung des zugesetzten Redoxindikators die Abfallsverzögerung des Potentials hervorgerufen wird und offenbar auch seine Kapazität daran beteiligt ist.

Alle diese Ergebnisse führen uns zu dem Schluss, dass das Potential der Hefesuspension unabhängig von der Gegenwart des Redoxindikators denselben definitiv eingestellten Endwert erreichen kann, obwohl betreffs des jeweiligen Potentialwerts in jedem beliebigen Zeitpunkt die Messungen, je nach dem sie mit oder ohne Redoxindikator durchgeführt werden, nicht immer in Übereinstimmung kommen, weil der zeitliche Verlauf der Potentialkurve durch den Zusatz des Redoxindikators eigentümlich beschwert wird.

## 8. DISKUSSION.

J. LEHMANN (1934) beobachtete das Potential des Einzelsystems der Gärung: Alkohol-Dehydrogenase-Acetaldehyd, was dabei als Oxydans zu dienen schien. F. LIPMANN (1934 c) hat die Versuche über die Bildung des Reduktans bei der Gärung des Mazerationssafts der Hefen potentiometrisch ausgeführt. Da aber die Gärung zu einem sehr komplizierten Vorgang gehört, so ist es dabei schwer zu versichern, welches von verschiedenen, an der Gärung vorliegenden Reaktionssystemen an der Feststellung des Potentials beteiligt sei.

In meinen Versuchen scheinen die ganzen Systeme zwar im reinen Stickstoff ins stabile Gleichgewicht gekommen zu sein, weil sich das Potential nach der vollständigen Ersetzung der im Gefäß befindlichen Luft durch den reinen Stickstoff auf einem definitiven Niveau einstellte und darauf lange Zeit konstant blieb. Das dabei erhaltene Potentialniveau war wesentlich negativ. Und der Endwert des Redoxpotentials der Hefesuspension war ja ganz gleich, unabhängig davon, ob man ihn

mit Hilfe der potentiometrischen Messung oder der Indikatormessung feststellen wollte.

B. C. J. G. KNIGHT (1930 a) mass bei Mikroben das Redoxpotential im sterilen Kulturmedium mit Hilfe der beiden genannten Methoden nebeneinander. Dabei wurde es gefunden, dass die beiden Verläufe des Potentials bei pH niedriger als 7,5 annähernd parallel miteinander waren, aber deren Endwerte nicht immer übereinstimmten und zwar bei Indikatormessung etwa 20 mV. niedriger als die bei den potentiometrischen waren. L. MICHAELIS (1929) schrieb folgendermassen: Ein bestimmtes, von der Natur des messenden Werkzeuges (Elektrode, Indikator) unabhängiges Potential ist nur dann zu erwarten, wenn das ganze System im Gleichgewicht steht. In meinen Versuchen, wobei das Elektrodenpotential der Hefesuspension in Gegenwart einer Spur von Redoxindikatoren neben den Veränderungen ihrer Farben beobachtet wurden, stimmte der Endwert des Elektrodenpotentials mit dem Potentialwert, welcher von der Entfärbung des zugesetzten Indikators aus zu beurteilen war, beinahe überein.

Auf Grund dieser Überlegungen kann man zu dem Schluss kommen, dass der wahre Endwert des Redoxpotentials der Suspension lebender Hefezellen bei  $\text{pH}=5,4$ , unter der anaeroben Bedingung, niedriger als die Normalpotentiale der angewandten Redoxindikatoren, d. h. Methylenblau, Indigotetrasulfonat, Indigotrisulfonat und Janusgrün bei der genannten Bedingung liegen muss, wenn er sich auch ganz nahe zu dem letzteren befindet.

Überdies unter der anaeroben Bedingung ist gar keine wesentliche Verschiedenheit zwischen den Endwerten der Redoxpotentiale sowohl aus der potentiometrischen als auch aus der Indikatormessung feststellbar.

Wenn man diesen Sachverhalt in Betracht zieht, so kann man sogleich erkennen, dass die in meinen Versuchen erhaltenen Resultate beinahe mit denen von E. AUBEL, E. AUBERTIN und L. GENEVOIS und auch von C. FROMAGEOT und P. DESNUELLE gleich gesetzt werden können, dagegen von denen von A. J. KLUYVER und J. C. HOOGERHEIDE stark abweichen. Diese Verschiedenheit ist um so mehr von grosser Bedeutung, als meine Versuche gerade wie diejenige von A. J. KLUYVER und J. C. HOOGERHEIDE potentiometrisch ausgeführt wurden. Es ist aber augenblicklich schwer zu sagen, warum meine Resultate von denen von A. J. KLUYVER und J. C. HOOGERHEIDE abweichen sollten.

Meiner Ansicht nach gibt es wenigstens zwei Möglichkeiten für das Zustandekommen dieser wesentlichen Verschiedenheit der beiden Resultate; nämlich erstens das Vorhandensein der Sauerstoffspur im Gefäss, was



auf die unvollkommene Ersetzung der Luft durch den Stickstoff oder zufällige Unreinheit des angewandten Stickstoffs zurückgeführt werden könnte, und zweitens der Einfluss der beschwerenden Wirkung des zugesetzten Methylenblaus auf den Potentialverlauf.

Was die Einwirkung der Sauerstoffspur auf das Redoxpotential betrifft, so scheint es durchaus möglich, dass wenn sich selbst eine Spur des Sauerstoffs im Gefäß befindet, so erstens direkt die reduzierten Teile des Gärsystems ziemlich schnell oxydiert und dementsprechend das Mengenverhältnis  $[Ox] : [Red]$  gar nicht kleiner werden kann, wodurch das Potential notwendigerweise das höhere Niveau zur Folge hat, und zweitens indirekt physiologische Änderung des Stoffwechsels der Hefezellen, weil die Atmung neben der Gärung vorgeht, die Einwirkung auf das Potential in der gleichen Richtung zu erwarten ist.

Meine schon beschriebene Beobachtung wies auch darauf hin, wie stark selbst eine Spur des Sauerstoffs auf das Redoxpotential einwirkt. L. MICHAELIS und L. FLEXNER (1928) beobachteten die störende Wirksamkeit aller kleinster Sauerstoff-Beimengungen bei der Messung des Cysteinopotentials. B. C. J. G. KNIGHT (1930) berichtete, dass das Potential im Mikroben haltigen Kulturmedium sowohl durch das Mischungsverhältnis zwischen dem sorgfältig gereinigten und dem ungereinigten Stickstoff als auch durch die Zufuhrgeschwindigkeit des gemischten Gases auf dem beliebigen Niveau beschwert werden konnte. Diese Tatsachen sprechen für die erste Möglichkeit.

Es bleibt noch eine andere Möglichkeit zu berücksichtigen, inwieweit der zugesetzte Redoxindikator das Potential im Kulturmedium auf einem bestimmten Niveau beschweren kann. Von R. K. CANNAN, B. COHEN und W. M. CLARK (1926) ist ein diesbezüglicher Versuch gemacht worden, wobei sie beobachteten, dass die Potentialkurve bei der Hefesuspension an dem Potentialbereich des zugesetzten Methylenblaus merklich abgeflacht wurde und sobald das beschwerend wirkende Methylenblau völlig reduziert wurde, das Potential wieder zu sinken fortsetzte. L. F. HEWITT (1933) beschrieb in seiner Monographie, dass als die beschwerende Wirkung des zugesetzten Redoxindikators schwach war, so wegen des ganz fehlenden oder geringen Einflusses des Indikators die Negativität des Potentials wieder weiter fortschritt; als aber seine Wirkung sehr stark war, das Potential ausserordentlich beschwert und ohne weitere Überschreitung aus seinem Potentialbereiche eingestellt wurde. Die Verzögerung des Potentialabfalls, welche durch die beschwerende Wirkung des Redoxindikators bei dem besonderen Potentialbereich hervorgerufen war, trat auch in meinem

Versuch auf. Dabei wurde auch Abhängigkeit der beschwerenden Wirkung von dem Kapazitätsfaktor des zugesetzten Indikators festgestellt, d. h. je geringer seine Kapazität war, desto schwächer war seine Wirkung.

Was nun die angewandte Methylenblaukonzentration (0,0001 %), womit auch A. J. KLUYVER und J. C. HOOGERHEIDE die Versuche ausführten, anbetrifft, so war die beschwerende Wirkung auf das Potential bezw. die Verzögerung an der Potentialkurve sehr schwach. Von der zweiten Möglichkeit, d. h. der beschwerenden Wirkung des zugesetzten Methylenblaus kann also, wenigstens für die genannte Konzentration, keine Rede sein.

Bei den Versuchen in dieser Richtung wurden früher die Redoxindikatoren dem Medium zugesetzt, um die Einstellungsgeschwindigkeit des Potentials zu erhöhen (B. ELEMA, A. J. KLUYVER und J. W. VAN DALFSEN (1934) und A. J. KLUYVER und J. C. HOOGERHEIDE (1934)). Dabei wurde gewöhnlich der im betreffenden Potentialbereich teilweise reduziert vorliegende Redoxindikator als der geeignete gewählt.

Man erinnere sich hier an das in Abschnitt 6 erhaltene Ergebnis. Bei Gegenwart des Janusgrüns, welches im betreffenden Potentialbereich teilweise reduziert wurde, war ziemlich kürzere Zeit als bei den anderen völlig reduzierten Redoxindikatoren erforderlich, um das bestimmte Potentialniveau zu erreichen. Ohne Indikator war aber die Einstellungsgeschwindigkeit trotzdem ähnlich gross wie in Gegenwart des Janusgrüns und wurde der stabile Potentialverlauf beobachtet. Es scheint deswegen wünschenswert, diese Frage noch weiter eingehend auseinanderzusetzen.

F. LIPMANN (1934 a, b) war der Meinung, dass die Resultate aus der potentiometrischen Messung ohne geeignete Indikatoren sehr unbefriedigend waren und erst in ihrer Gegenwart eine charakteristische Potentialkurve gefunden werden konnte. Wenn man aber in Betracht zieht, dass die potentiometrische Messung tatsächlich die einzige Methode ist, welche ohne Einwände von den beschwerenden, katalytischen und auch giftigen Wirkungen der Redoxindikatoren bei der Indikatormessung das Redoxpotential im biologischen System feststellen lässt, so muss bei der potentiometrischen Messung in Gegenwart von Redoxindikatoren eine genügende Vorsicht auf die Indikatorenwirkungen verwendet werden.

## 9. ZUSAMMENFASSUNG.

1. Durch die Anwendung des WARBURGschen manometrischen Apparats, in dessen Gärungsgefäß eine Platinelektrode, ein KCl-Agarheber und ein

Gaszufuhrrohr eingebunden waren, wurden in den Suspensionen lebender Hefezellen (*Saccharomyces cerevisiae*) die Redoxpotentiale gemessen und gleichzeitig die von der Gärung entwickelten Kohlensäuremengen festgestellt.

2. Unter streng anaerober Bedingung ist der Endwert des Redoxpotentials der glukosehaltigen, auf  $\text{pH}=5,4$  gepufferten Hefesuspension etwa von  $-170$  bis  $-180$  mV. festgestellt.

3. In den genannten Hefesuspensionen, denen eine geringe Menge der Redoxindikatoren zugesetzt war, wurden die Potentialverläufe untersucht und daneben die Entfärbung der zugesetzten Indikatoren verfolgt. Von den angewandten Indikatoren bewirkten Methylenblau, Indigotetra-sulfonat und Indgotrisulfonat in ihren eigentlichen Potentialbereichen infolge der beschwerenden Wirkungen die Abfallsverzögerungen der Potentiale, und sie wurden vollständig reduziert; Janusgrün verhält sich aber ganz anders, und es wurde im betreffenden Potentialbereich nicht vollständig reduziert.

4. Es wurde festgestellt, dass das Potential der betreffenden Hefesuspension unabhängig von der Gegenwart des Redoxindikators einen bestimmten Endwert erreicht.

5. Mit verschiedener Konzentration des zugesetzten Methylenblaus,  $1 \cdot 10^{-4}\%$  und  $5 \cdot 10^{-4}\%$ , wurde sichergestellt, dass die Abfallsverzögerung des Potentials, welche auf die beschwerende Wirkung dieses Indikators zurückzuführen war, länger bei der letzteren als bei der ersteren war.

6. Unter streng anaerober Bedingung war immer der beinahe gleiche Endwert des Redoxpotentials der Hefesuspension sichergestellt, unabhängig davon, ob die Potentialmessungen potentiometrisch oder indikatorisch ausgeführt werden.

Zum Schluss möchte ich meinem hochverehrten Lehrer, Herrn Professor Dr. Y. YAMAGUTI, unter dessen Leitung vorliegende Arbeit ausgeführt wurde, für die hilfreiche Unterstützung und das rege Interesse meine herzlichen Dank aussprechen.

#### LITERATURVERZEICHNIS

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# COMPARISON BETWEEN THE FERTILIZATION PERCENTAGE AND HEART PULSATION OF JAPANESE OYSTERS AFFECTED BY SALINITY, KCl, MgCl<sub>2</sub> AND CaCl<sub>2</sub><sup>1,2)</sup>

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(With eleven figures)

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The present work was undertaken as a re-investigation of a previous work (HAMADA, 1933) on the correlation between fertilization and cardiac activity in oysters, and was primarily concerned with the effects of dilution of sea water and of chlorides of potassium, magnesium and calcium in artificial media.

In the previous paper, it was shown that in various experimental media there seems to be a certain general correspondence between variations in fertilization of eggs and those in activity of isolated heart as indicated by the product of amplitude and frequency of its pulsation. However, in later experiments on the effect of KCl in *Ostrea circumpicta*, a much closer parallelism was found between fertilization percentages and variations in amplitude of heart beating than between the former and the products of amplitude and frequency. Moreover, in the previous work, heart beat and fertilization were not observed in the same individuals, but observed separately in different specimens, and sometimes even in different sexes. In the present investigation, in order to give more complete data on this line, the heart beat was observed only in these mature female oysters, the fertilization of eggs from which was examined in the media of the same chemical composition and under the same temperature conditions. The results of experiments are presented below.

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#### MATERIALS AND METHODS

*Ostrea circumpicta* PILSBRY has served as the chief experimental material but has been supplemented by *O. rivularis* GOULD for certain comparative purposes. The specimens of *O. circumpicta* from the Mutsu Bay were tested at Asamushi Marine Biological Station of Tôhoku Imperial University, and most of the specimens of the same species from the south-west coast of Honshu were examined at Kaike, Hôki Province, and the remainder part at Fukuoka. The latter species was collected from the Sea of Ariake and examined at the Biological Laboratory of Fukuoka Kôtô-Gakko (Fukuoka Junior College).

As the experimental media for the present investigation, the several different dilutions of natural sea water and various modifications of BERGER's solution were employed. In this work the concentration or salinity of normal and diluted sea water was indicated in terms of specific gravity at 15°C. Controls in sea water of 1.023 in specific gravity were used, since in this concentration of sea water the percentage of fertilization of eggs from both species is constantly high and almost 100 per cent, and the isolated heart of the oysters shows normal pulsation in rhythm and contraction.

In tentative experiments, VAN'T HOFF solution was found not to be adequate as an artificial balanced solution for both egg and heart of these species, although isolated heart *O. circumpicta* in this solution is said to continue indefinitely its rhythmical pulsation (TAKATSUKI, 1927). In VAN'T HOFF solution the fertilization percentage of eggs is generally much less and the frequency of heart beating is greater than in natural sea water (HAMADA, 1929, 1933). And it was also found that a solution devised by BERGER (1929) was much satisfactory for these forms. The calcium-potassium ratio in that solution is much closer to that of sea water than in VAN'T HOFF solution, but even in this solution the amplitude

is still less and frequency is somewhat greater than in natural sea water. In view of these results, modified BERGER solutions were made up on the basis of two formulas as follows:

#### Solution A

NaCl .....	86.5 cc.
CaCl <sub>2</sub> .....	2.3 cc.
KCl .....	1.5 cc.
MgCl <sub>2</sub> .....	6.0 cc.
MgSO <sub>4</sub> .....	3.7 cc.

#### Solution B

NaCl .....	83.0 cc.
CaCl <sub>2</sub> .....	2.3 cc.
KCl .....	1.5 cc.
MgCl <sub>2</sub> .....	9.5 cc.
Na <sub>2</sub> SO <sub>4</sub> .....	3.7 cc.

All solutions were composed of 4/10 molar concentration. The mixture was raised to pH 8.2 by addition of a minute quantity of NaHCO<sub>3</sub>. The specific gravity of these solutions is about 1.021.

These standard solutions were found to be most satisfactory, so far as the writer's trials were concerned, in which the fertilization percentages of eggs and pulsation of isolated heart of oysters were approximately those in natural sea water (1.023 in specific gravity). The solution A was adopted as a standard solution for the examination of the effects of excess and deficiency of KCl and of CaCl<sub>2</sub>, and the solution B, for that of MgCl<sub>2</sub>. The variation in the amount of each salt to be tested were given in volume per cent, that is, in cubic centimeters, of 4/10 molar solution of it; the remaining part of the solution being essentially the standard solution but with the removal of the salt in question.

The artificial fertilization of eggs was made in ordinary way in normal sea water and in the solutions to be tested, and the fertilization percentages, the fertilization ratio relative to the percentage in normal sea water was calculated for each experimental medium, and was indicated by per cent of the control which was taken as 100.

The pulsations of the isolated heart of each oyster were recorded on a drum of kymograph by a single suspension method. The heart was immersed in normal sea water until the pulsation had become well established; then it was exposed to the solution to be tested until the character



of the response became steady, for which usually it took 7 to 8 minutes after exposure; after which, to restore its beating, it was again immersed in the natural sea water. From these records, the ratios of amplitude and of frequency of heart beating in experimental media relative to those in normal sea water were determined respectively, as a per cent of the latter. And the relative amounts in product of amplitude and frequency were calculated from these data. The results of the experiments are presented in a summarized form and graphed in Figures.

### EXPERIMENTAL RESULTS

*Effects of diluted sea water.* The diluted sea water in specific gravity from 1.020 down to 1.005 were tested; and controls in normal sea water, specific gravity of which was 1.023, were used.

The experimental results of the experiments on *O. circumpecta* are given in Figures 1 to 3, with percentages of ratios in fertilization (solid circles), amplitude (circular outlines) and frequency (crosses) of heart beating and their product (triangles) as ordinates against specific gravities of sea water used as abscissae. Figure 1 shows the data obtained from

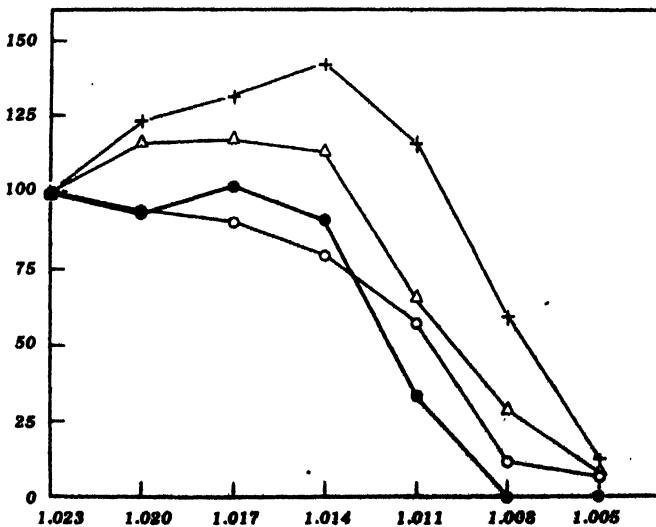


Fig. 1. The relation of variations in fertilization percentage of eggs (solid circles), and amplitude (circular outlines) frequency (crosses) of heart pulsation, and of those in the product of amplitude and frequency (triangles) to the dilution of sea water in *O. circumpecta* from Mutsu Bay. Ordinates represent percentages relative to the controls in normal sea water.

the experiments at Asamushi on the Mutsu Bay material, and Figure 2 and 3, those from the experiments at Kaike and Fukuoka on the Hôki specimens. The data given in these graphs are based on the averages of the results of the experiments of 10 lots for each Figure.

As shown by the graphed data in Figure 1, the fertilization ratios (solid circles) in Mutsu Bay material of *O. circumpecta* are nearly as high as the control in the normal sea water and show a little irregularity within the limits (85.3 to 101.9) in less diluted sea water, specific gravity of which ranges from 1.020 to 1.014, but it decreases in more diluted sea water, and no fertilization occurs at sp. gr. 1.008. Relative ratio of the amplitude of heart beating (circular outlines) shows a continuous decrease with the decrease in specific gravity, while ratio of the frequency of pulsation (crosses) increases with a decrease in specific gravity; and reaches a maximum, 142.2, at sp. gr. 1.014; then decreasing with increase in dilution. In consequence, in the Mutsu Bay specimens of *O. circumpecta*, the relation of variation in fertilization ratio to the concentration of sea water is rather closely parallel to that of the amplitude than to that of the frequency of heart pulsation.

Figure 2 shows the results of experiments at Kaike on the Hôki specimens of the same species. In these specimens, fertilization ratios at the specific gravities of sea water, 1.020, 1.017 and 1.014 are approximately the same, or slightly higher (100.5 to 105.2 per cent of the control) than the control in the normal sea water; but in more diluted sea water it decreases with an increase in dilution. In these specimens, like the specimens from the Mutsu Bay, the amplitude ratio decreases

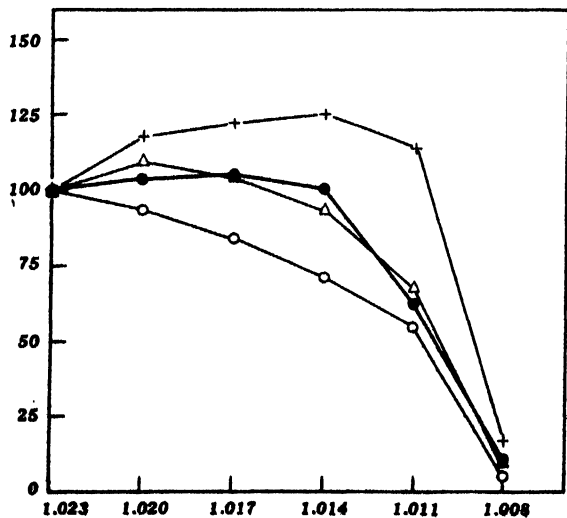


Fig. 2. The relation of variations in fertilization percentage of eggs, amplitude and frequency, and of those in the product of amplitude and frequency to the various dilutions of sea water in *O. circumpecta* from Hôki (Kaike examination). Ordinates, abscissae, and symbols, as in Fig. 1.

continuously with the decreasing of the specific gravity, but the frequency ratio increases and reaches a maximum (124.8), at sp. gr., 1.014, then it decreases in much more diluted sea water. Accordingly, in the Hôki specimens examined at Kaike, the fertilization ratio varies neither with the variation of the amplitude nor with that of the frequency of heart beat, but, as the graph shows, it seems to vary with the variation in product of amplitude and frequency (triangles, in Fig. 2).

However, in the Hôki specimens examined at Fukuoka, the data of which are graphed in Figure 3, the variations in the fertilization ratio in diluted sea water are more closely related to those in the amplitude than to the frequency variations or to those in product of amplitude and frequency. The frequency ratio of these specimens highly increases up

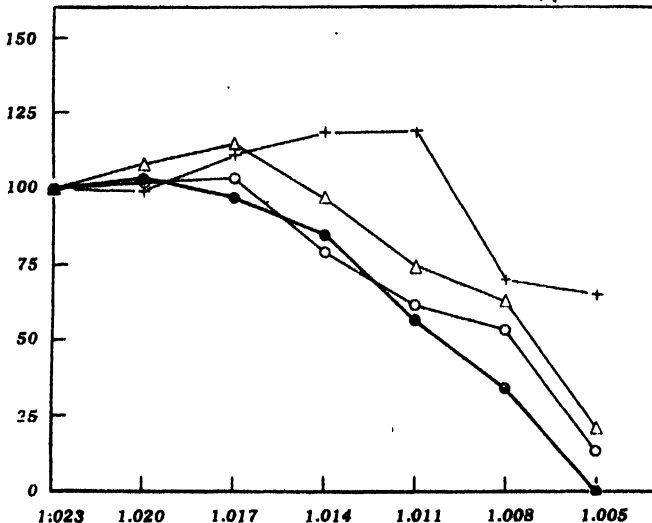


Fig. 3. The relation of variations in fertilization percentage, amplitude and frequency, and of those in product of amplitude and frequency to the dilution of sea water in *O. circumpicta* from Hôki (Fukuoka examination). Ordinates, abscissae, and symbols as in Fig. 1.

to 119.3 at sp. gr. 1.011, while at the same specific gravity, both fertilization ratio and amplitude ratio decrease down to 56.6 per cent and 61.5 per cent, respectively. And the frequency ratio is still relatively high (65.2), even at sp. gr. 1.005, at which fertilization of eggs is completely inhibited and the amplitude ratio is only 13.5.

In consequence, among the results from these three experiments on *O. circumpicta*, there are found some differences in correlation of the

fertilization ratio to the heart activity, but for which no definite reason can be given, since the further analytical data necessary for account are not at hand. However, from comparison of the graphs in Figures 2 to 4, it may be permissible to conclude that in this species the fertilization ratio varies in general with dilutions of sea water, and its variations run closely parallel either to the variations in amplitude (Fig. 1, 3), or with those in product of amplitude and frequency of heart pulsation (Fig. 2).

*Effect of variations in amount of KCl in artificial medium.* In this series, the experiments were performed in artificial solution A (p. 575), in which the volume percentage of 4/10 molar solution of KCl was changed as follows: 0 per cent (i. e., complete lack of KCl), 2 per cent, 4 per cent, 6 per cent, 8 per cent, 10 per cent and 12 per cent, but without any change in the concentration of the other salts; and the controls in natural sea water, specific gravity of which was 1.023, were employed. The results of the experiments on Mutsu Bay specimens of *O. circumpecta*, are presented in Figure 4, and those on Hôki specimens, in Figure 5. In graphs, solid circles represent fertilization ratios; circular outlines, amplitude ratios; crosses, frequency ratios of heart beat; and triangles, ratios in product of the latter two; abscissae represent concentrations of KCl in volume per cent in the artificial solution A. The data given in Figures 5 and 6 are based on the averages from the results of experiments on each 10 individuals, respectively.

In *O. circumpecta* (Figs. 4, 5), the relations of variations in fertilization ratio and in amplitude ratio of heart beating to the concentrations of KCl are generally the same in the specimens from both localities, Mutsu Bay and Hôki. In other words, these ratios in KCl-free solution decrease more or less, and they show some maximal values at 4 per cent of KCl, but decrease with the increase in concentration of KCl in the solution. Regarding the effect on the frequency of heart pulsation, however, there is a great difference between the two above mentioned species. In Mutsu Bay specimens, the frequency ratio (crosses in Fig. 4) alters quite irregularly: it is considerably high (129.1) at 0 per cent of KCl, and decreases to 108.9 at 4 per cent, but greatly increases, (139.2) at 8 per cent, and again decreases down to 70.9 at 12 per cent, while in Hôki specimens (crosses in Fig. 5), though it is also exceedingly high, (150.5) in KCl-free solution, it decreases continuously as the concentration of KCl increases. Therefore, from these results, it seems impossible to draw any simple definite view concerning the effect of various concentrations of KCl upon the frequency of heart beat of these species of oyster, with exception of

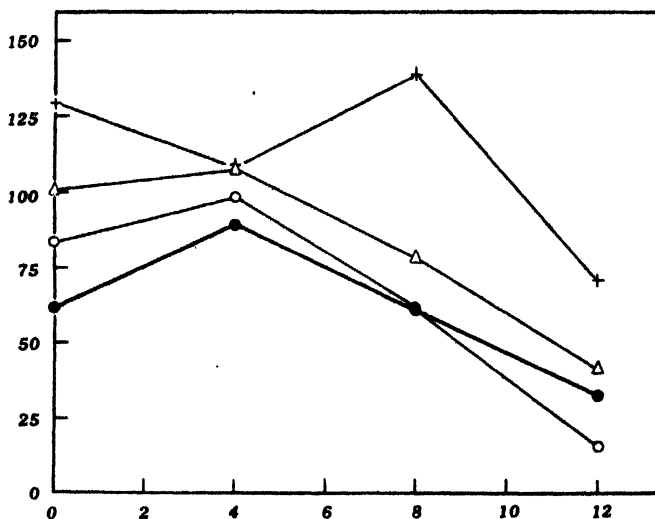


Fig. 4. The variations in fertilization percentages of eggs (solid circles), amplitude (circular outlines) frequency (crosses) of heart beat, and those in the product of amplitude and frequency (triangles) in different concentrations of KCl in modified BERGER solution in *O. circumpicta* (Mutsu Bay specimens). Ordinates percentages relative to the controls in the normal sea water; abscissae volume percentages of 4/10 molar solution of KCl.

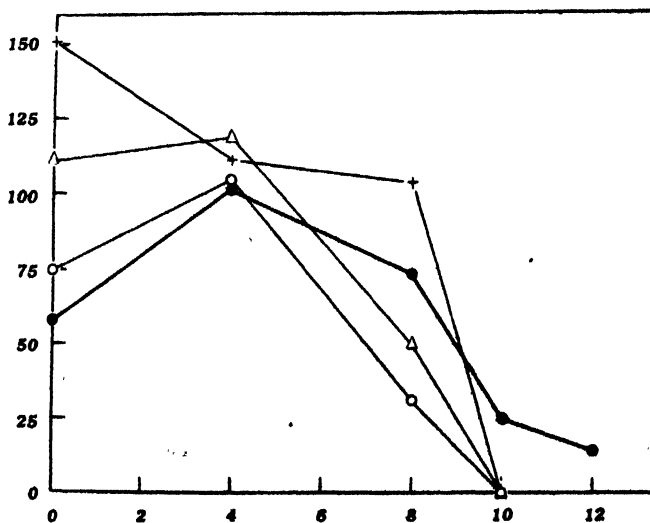


Fig. 5. The variations in fertilization percentage, amplitude and frequency and those of the product of amplitude and frequency of heart beating in different concentrations of KCl in *O. circumpicta* (Hôki specimens). Ordinates, abscissae and symbols, as in Fig. 4.

the fact that, in the solutions with much more quantity of KCl than 4 to 6 per cent, it decreases with increase in amount of KCl.

*Effect of variations in amount of  $MgCl_2$  in artificial medium.* In this series, *O. circumpicta* from both localities, Mutsu Bay and Hôki, was examined with solutions containing different concentrations of  $MgCl_2$  in a standard solution B (p. 575). The concentration was also given in volume percent of 4/10 molar solution of the salt, as in the previous cases with KCl. The percentages used here were as follows: 0 per cent (i. e., complete lack of  $MgCl_2$ ), 10 per cent, 20 per cent and every 10 per cent increase up to 70 per cent. The results of the experiments on Mutsu Bay specimens are graphed in Figure 6 and these on Hôki specimens, Figure 7. These graphed data are based on the average from the results of experiments of 10 individuals for each Figure.

In Mutsu Bay species, fertilization ratio (solid circles in Fig. 6) is a little lower (87.9) at 0 per cent than in normal sea water, and slightly

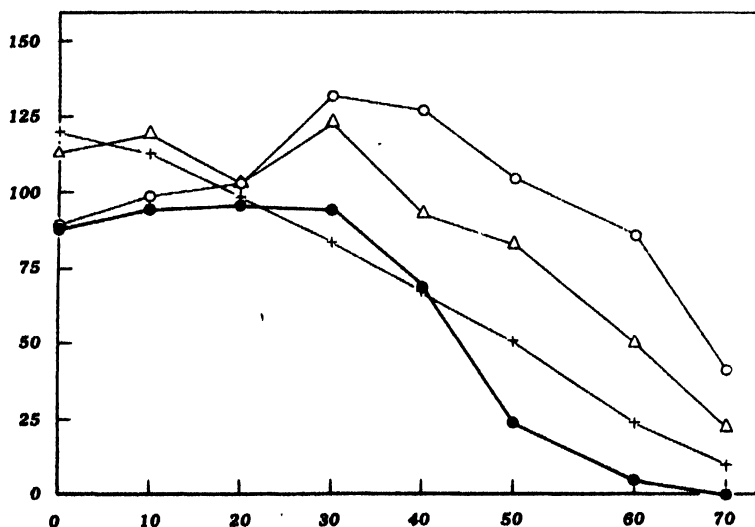


Fig. 6. The variations in fertilization percentage, amplitude and frequency of heart pulsation and those in the product of amplitude and frequency in different concentrations of  $MgCl_2$  in modified BERGER solution in *O. circumpicta* from Mutsu Bay. Ordinates percentages relative to the controls in the normal sea water, abscissae volume percentages of 4/10 molar solution of  $MgCl_2$  in the media. Symbols, as in preceding Figures.

increases (94.4, 95.7, 94.3) at 10 to 30 per cent of  $MgCl_2$ , but above these concentrations, it decreases with the increase in concentration of

the salt. The amplitude ratio (circular outlines) in these specimens, like their fertilization ratio, shows rather low value (88.5) at 0 per cent, and it increases high up to 131.9 at 30 per cent, but decreases gradually with the further increase of  $MgCl_2$ .

Fertilization ratio of Hôki specimens (Fig. 7) is also low (79.8) in  $MgCl_2$ -free solution, and it rises up to the normal ratio (99.3) at 10 per cent of  $MgCl_2$ , but it decreases as the concentration of the salt increases in the media. The amplitude of heart beat in these specimens is low

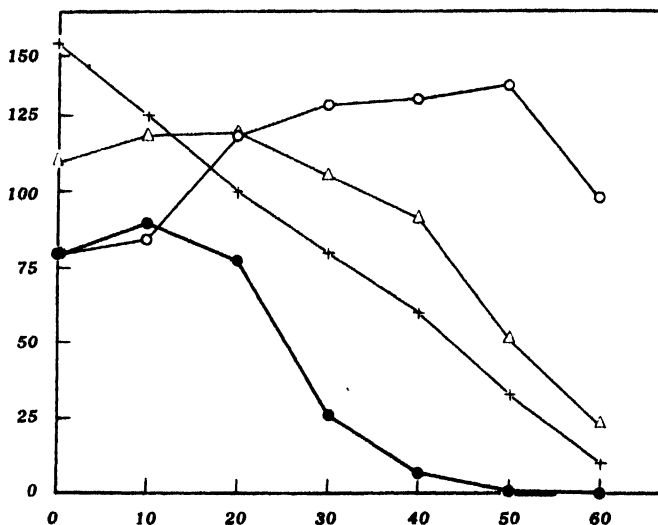


Fig. 7. The variations in fertilization percentage, amplitude and frequency of heart beat and those in the product of amplitude and frequency in different concentrations of  $MgCl_2$  in modified BERGER solution in *O. circum-picta* from Hoki coast. Ordinates, abscissae and symbols, as in Fig. 6.

(79.0) at 0 per cent, and increases up to 135.0 at 50 per cent, then falls again at 60 per cent of the salt. The frequency ratio of heart pulsation in both, Mutsu Bay and Hôki specimens, is much higher (122.8 and 149.5) at 0 per cent than the controls in natural sea water, and contrarily to the depression of amplitude ratio, it shows a continuous increase with the increase in concentration of  $MgCl_2$  salt. From graphs in Figures 6 and 7, it is seen that the fertilization ratio in *O. circum-picta* increases a little at the concentration of  $MgCl_2$  from 0 to 10 per cent, and goes parallel to the increase in amplitude, but in further concentration  $MgCl_2$  it decreases, running parallel to the decrease in frequency ratio.

*Effect of variations in amount of  $CaCl_2$  in artificial medium.* In this

series, *O. circumpicta* was examined in a standard solution A (p. 575) in which the concentration of  $\text{CaCl}_2$  was altered from 0 to 80 per cent in volume. The results of experiments are given in graphs in Figures 8 and 9. The graphed data for Figure 8 are based on averages of the results obtained from the experiments on 10 individuals, and those for Figure 9, from the experiments on 12 individuals.

In Mutsu Bay specimens (Fig. 8) the fertilization ratio is remarkably low (35.9) in  $\text{CaCl}_2$ -free solution, and it nearly recovers the normal ratio (90.3) at 10 per cent of  $\text{CaCl}_2$ , but decreases again in further increase

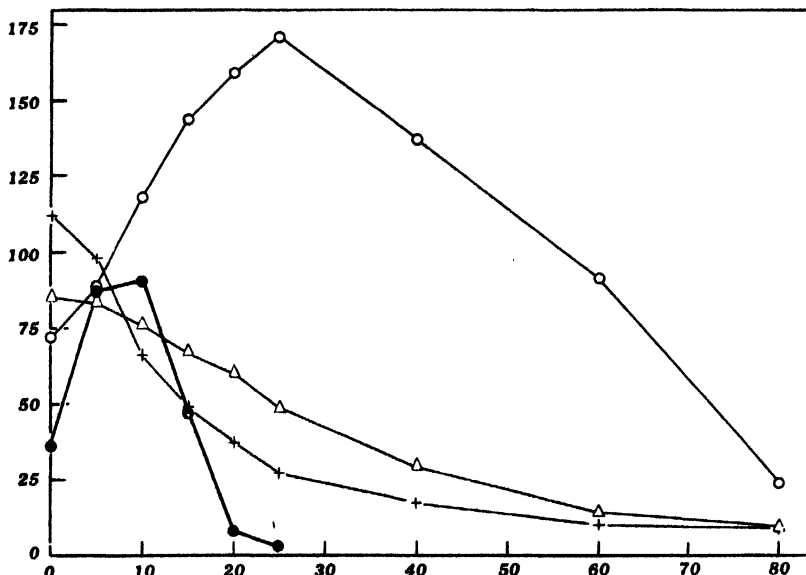


Fig. 8. The variations in fertilization percentage of eggs, the amplitude and frequency of heart beat and those in the product of amplitude and frequency in different concentrations of  $\text{CaCl}_2$  in modified BERGER solution in *O. circumpicta* from Mutsu Bay. Abscissae volume percentages of 4/10 molar solution of  $\text{CaCl}_2$ ; ordinates and symbols, as in the preceding Figures.

in concentration of the salt, and almost no fertilization occurs at 25 per cent. The amplitude ratio in Mutsu Bay specimens is also relatively low (72.5), and in higher concentration, it increases to a great extent, reaching a maximum (171.1) at 25 per cent of the salt, but beyond this concentration, it shows a uniform decrease, with increase in amount of  $\text{CaCl}_2$  in the solution. The frequency ratio is a little higher (111.9) at 0 per cent than the control, and as the concentration of the salt rises up in the media; it continuously decreases down to 9.6 at 70 per cent.



Concerning relations of fertilization ratio and amplitude ratio to the concentration of  $\text{CaCl}_2$ , Hôki specimens (Fig. 9) appear to be nearly the same as in Mutsu Bay specimens, but a maximal variation of amplitude

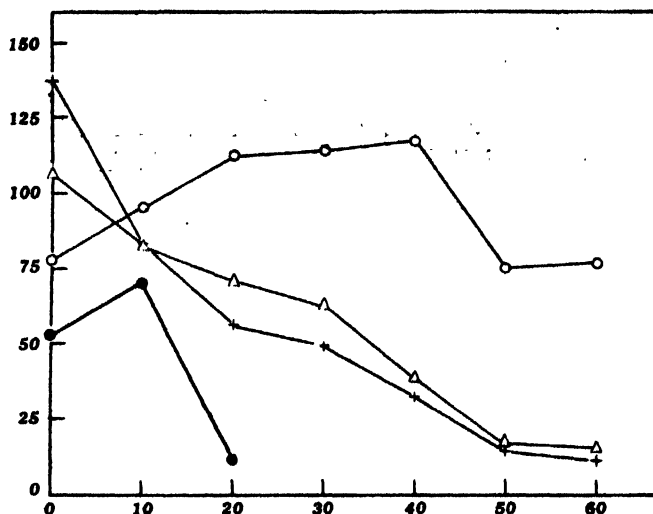


Fig. 9. The variations in fertilization percentage, amplitude and frequency of heart beat, and those in the product of amplitude and frequency in different concentrations of  $\text{CaCl}_2$  in modified BERGER solution in *O. circumpicta* from Hôki Coast. Ordinates, abscissae and symbols, as in Fig. 8.

at 40 per cent is much less (117.3) than that of the former (171.1) at 25 per cent. The frequency ratio at 0 per cent is exceedingly high (137.1) in Hôki specimens as compared with that in Mutsu Bay material, and like those in Mutsu Bay ones, it decreases with an increase in concentration of  $\text{CaCl}_2$ , reaching very low ratio (11.2) at 60 per cent.

*Experiments on O. rivularis with diluted sea water and different concentrations of KCl in modified Berger solution.* The experiment with *O. rivularis* was by far less extensive than with *O. circumpicta*, and concerned only with diluted sea water and KCl salt. But for the purpose of comparison the data obtained are appended here.

The experimental results on *O. rivularis* with diluted sea water are graphed in Figure 10. These graphs are based on the averages from the results of 8 lots of experiments, each consisting of eggs and a heart from a single oyster. In this species, fertilization percentage (solid circles) is not affected by such dilutions of sea water as 1.020 to 1.011 in specific gravity, and it shows there nearly 100 per cent of the control (97.8 to

99.0). It decreases considerably in much lower concentrations, i. e., 44.0 at the specific gravity 1.008, and no fertilization occurs at sp. gr. 1.005.

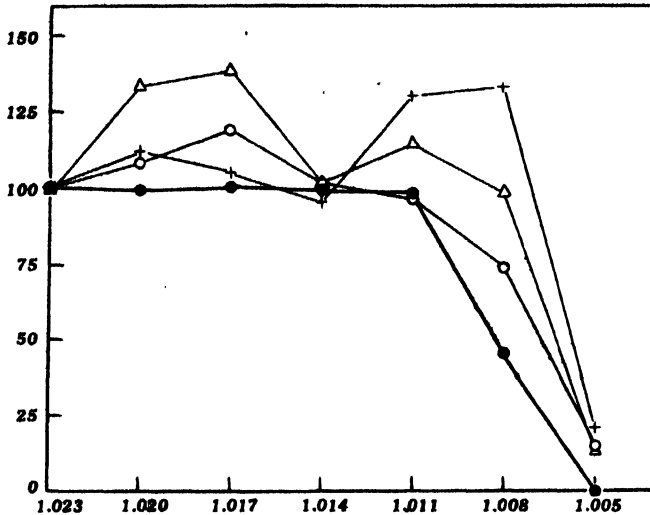


Fig. 10. The variations in fertilization percentage of eggs, amplitude and frequency of heart beat, and those in the product of amplitude and frequency at different specific gravities of sea water in *O. rivularis*. Ordinates, abscissae and symbols, as in Fig. 1.

Accordingly, the fertilization ratio is almost constant at sp. gr. 1.020 to 1.011 and as high as the control in normal sea water at sp. gr. 1.023, but in more diluted sea water it decreases with an increase in dilution. Relative ratio of the amplitude of pulsation (circular outlines) in isolated heart increases a little with increase in dilution, reaching a maximum, 119.2 at sp. gr. 1.017, and it comes again to the same amplitude as the control, at sp. gr. 1.011, then it decreases as the specific gravity also decreases. However, relation of frequency ratio with the concentration of sea water is quite irregular. The frequency ratio (crosses) shows two maxima at sp. gr. 1.020 (112.5) and at sp. gr. 1.011 (133.3), and two minima at sp. gr. 1.014 (94.8) and at sp. gr. 1.005 (20.8). Therefore, as the graph shows, in *O. rivularis* variations of fertilization ratio in diluted sea water may be said to run parallel to those of the amplitude ratio, but not to these of frequency ratio, and, consequently not to those in product of amplitude and frequency of heart beating.

The data from the experiments with different concentrations of KCl in *O. rivularis* are graphed in Figure 11. The graphed data are based

on the averages from the results of experiments on 4 individuals. The fertilization ratio (solid circles) in KCl-free solution is considerably low, 73.8, and it increases, as the amount of KCl increases, reaching a maximum, 111.3, at 4 per cent of 4/10 molar KCl in the solution, but it gradually decreases with further increase in concentration of KCl. In KCl-free

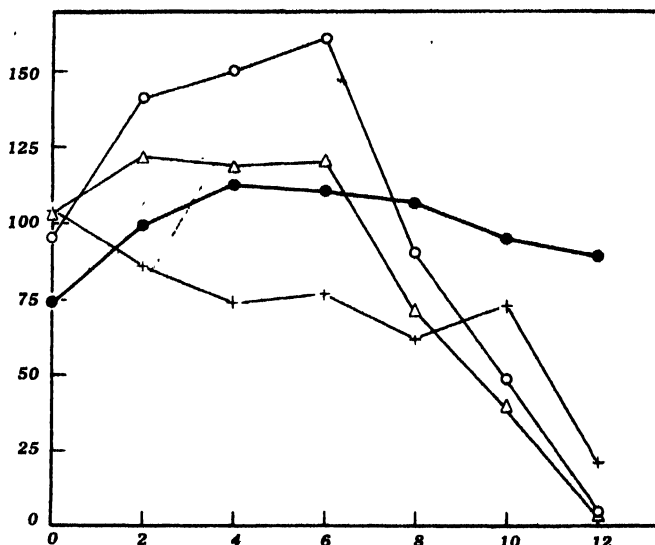


Fig. 11. The variations in fertilization percentage of eggs, amplitude and frequency of heart pulsation and those in the product of amplitude and frequency in different concentrations of KCl in modified BERGER solution in *O. rivularis*. Ordinates, abscissae and symbols, as in Fig. 4.

solution, the amplitude ratio (circular outlines) was not appreciably altered, i. e., 95.1 and as the concentration of KCl increases, it greatly increases to 141.5 at 2 per cent, 151.2 at 4 per cent, 160.1 at 6 per cent, but suddenly decreases at 8 to 12 per cent of KCl in the solution. That is to say, although in its general form the amplitude curve resembles the curve of fertilization, the range of variations in the former is much greater than in the latter. This means that, in this species, amplitude of heart pulsation is more greatly influenced by the action of KCl or K-ion than the fertilization. On the contrary, the frequency of heart beat (crosses) in KCl-free solution was not much affected, but it decreases almost continuously down to 20.8 at 12 per cent of KCl. The composition of the used solution, which contains 2 per cent of 4/10 molar solution of KCl, resembles pretty well that of the BERGER solution, though the relative

concentration of KCl is 1.8 per cent instead of 2.0 per cent. In this solution, the fertilization ratio of *O. rivularis* is practically the same (99.6) as in normal sea water, but amplitude of heart beat is considerably higher (141.5) and the frequency is somewhat lower (85.9) than the controls. These results well agree with those from the tentative experiments with VAN'T HOFF solution and BERGER solution (p. 575) in supporting the view that the latter two solutions are not satisfactory for heart and eggs of this species of oyster, especially with respect to the calcium-potassium ratio in the media. As to the parallelism between fertilization ratio and amplitude ratio, *O. rivularis* differs from *O. circumpecta* in their relative change in various concentrations of KCl. In *O. circumpecta* (Figs. 4 and 5), they vary rather closely with each other in each concentration, while in *O. rivularis*, the amplitude ratio is conspicuously higher at 2 to 6 per cent and exceedingly lower at 10 to 12 per cent of KCl than the fertilization ratio at the corresponding concentrations of KCl in the media.

#### GENERAL CONSIDERATION AND CONCLUSIONS

At the time of a previous communication (HAMADA, 1933), the writer held the opinion that in two species of oyster, *Ostrea rivularis* and *O. circumpecta*, the variation in fertilization percentage of eggs caused by either deficiency or excess of potassium, magnesium and calcium salts in an artificial medium and in natural sea water runs parallel to the variation in the product of the amplitude and frequency of heart pulsation. In some way, the present study seems to call this view in question, since, as mentioned above, in some cases the fertilization percentage has been found to be much more closely related to the variation in the amplitude of heart beat than to the variation in the product of amplitude and frequency.

The data of the present paper show that, in various dilutions of sea water, the fertilization percentage in these oysters varies most closely with the variation in the amplitude height (Figs. 1, 2 and 4), excepting the results of the experiments at Fukuoka on the Hôki specimens of *O. circumpecta* (Fig. 3), in which it varies with the variation in the product of amplitude and frequency. But as regards the effects of different concentrations of KCl,  $MgCl_2$  and  $CaCl_2$  in artificial media upon these relations between fertilization and heart function, it seems much complicated.

In the case of complete deficiency of any one of these three salts, the fertilization percentage and the amplitude of heart beat decrease

more or less, while the frequency generally increases. The grades of these variations are, of course, different not only in different species, but in different salts, which are withdrawn from the artificial balanced solutions. In most cases, the increase in concentration of any one of these salts in the medium causes a decrease in the frequency of heart beat almost continuously, but in Mutsu Bay specimens of *O. circumpicta* it shows quite an irregular variation with respect to the increase in amount of KCl (Fig. 4). With an increase in amount of the salts, both fertilization percentage and amplitude generally increase and reach maxima at certain concentrations, but again decrease in further concentration. However, even in these cases, the variation in fertilization does not always run parallel to the variation in the amplitude (Figs. 7, 8, 9 and 11). In *O. circumpicta* the fertilization percentage varies rather closely to the variation in the amplitude at different concentrations of KCl (Figs. 4 and 5), but in *O. rivularis*, both the increase and decrease in fertilization percentage are rather slight as compared with the marked variations in the amplitude at the corresponding concentrations of the salt (Fig. 11). As to the effects of excess of  $MgCl_2$  and  $CaCl_2$ , the parallelism between fertilization percentage and the amplitude height are kept only within the limit of relatively low concentration (ca. 0 to 10 per cent), and beyond this limit the former shows a continuous decrease with an increase in the concentration of these salts, while the latter still increases up to a certain amount. In consequence, when the amount of  $MgCl_2$  or  $CaCl_2$  in the solution is raised up over such limit concentrations, fertilization percentage of *O. circumpicta* decreases, and its variation comes to run parallel to the decrease in the frequency of heart beat (Figs. 5 to 9).

Thus, the effects of KCl,  $MgCl_2$  and  $CaCl_2$  in modified BERGER solutions on the fertilization are not always in complete accord with the effects of these salts on the heart activity. The chief differences found in *O. circumpicta* are first that the deficiency of the salts causes the decrease in the fertilization percentage and the increase in the frequency of heart beating; and second that excess of the salts generally results in decrease in the fertilization percentage, while the larger excess of  $MgCl_2$  and  $CaCl_2$  causes greater or less increase in the amplitude. As the results from these differences, in some solutions the fertilization percentage appears to vary closely parallel to the amplitude variation, and in other solutions, to the frequency variation.

However, if regardless of the minor differences, in most of the foregoing Figures the general form of the graphs showing the variation in

the product of amplitude and frequency of heart beat may be found to bear generally a rather well resemblance to that of the fertilization variations. Accordingly, the conclusion given by the writer in the previous paper (HAMADA, 1933) seems still good. In other words, roughly speaking, the relation of the fertilization of oyster eggs to the dilutions of sea water and to the concentrations of the salts in artificial media seems to correspond considerably well with that of the variation in the product of amplitude and frequency of the heart beating. In conclusion, beside the question relating to the cause of the effects of these factors, and of their variations and correlations, it is of much interest to suggest that in these mentioned above solutions, the variation in fertilization percentage has a certain close parallelism in a way or other to the variation in the heart activity in oysters.

As to the influence of the salinity of sea water upon the fertilization of oyster eggs, AMEMIYA (1928) reported that the optimum specific gravity (at 0°C.<sup>1)</sup>) for *O. rivularis* ranges 1.0153 to 1.0200 and for *O. circumpicta*, 1.0233 to 1.0281, and the minimal specific gravity necessary for development is 1.0056 in the former species and 1.0112 in the latter. The results of the present paper agree well with those given by AMEMIYA in supporting the view that developmental processes in *O. rivularis* can stand in much lower salinity than that in *O. circumpicta*. According to AMEMIYA, the salinity of the natural habitat of *O. rivularis* ranges from 9 to 30‰ (i. e., 1.0061 to 1.0222 in specific gravity at 15°C.), while that of *O. circumpicta* from 28 to 35‰ (i. e., 1.0206 to 1.0260 in sp. gr. at 15°C.). In fact, the specific gravity of sea water off Asamushi was found to be rather high and to show a slight variation, i. e., 1.02416 to 1.02505, through a year (1926), except in April (1.02171) and May (1.0229) when the melting snow on mountains in this district causes temporarily a little lower salinity of water (HATAI and KOKUBO, 1928). In the writer's observations at the years of 1927 to 1928, the specific gravity of water of Ariake Sea, where is the habitat of *O. rivularis*, varied from 1.007 to 1.021 at two miles off Okinohata, Chikugo Province, and near the estuary of the Okinohata River it was only 1.003 to 1.007 even in February, a month of minimal rain-fall of the year in this locality (HAMADA, 1933). In consequence, as has been suggested by AMEMIYA, the above mentioned differences in the susceptibility of eggs between two species are highly probable to have a intimate relation to the difference in ecological conditions of their habitats.

<sup>1)</sup> The specific gravities cited here from AMEMIYA's paper are those at 0°C.

According to the recent work by WALZL (1937) on an American species, *O. virginica*, the response of the heart to the excess and deficiency of KCl salt in VAN'T HOFF solution differs from that of *O. circumpecta* to those in the modified BERGER solution. In *O. virginica*, there is an optimum concentration of KCl in VAN'T HOFF solution, and either increase or decrease in this concentration results in decrease in the amplitude, and increase in the frequency to some extent, but the latter decreasing in further excess or deficiency of the salt. However, concerning the response to  $\text{CaCl}_2$  salt, the variations in the amplitude and frequency of heart pulsation in *O. virginica* are generally in accord with those of *O. circumpecta*, except to the extreme low concentrations of the salt. The amplitude shows a continuous increase with increase in concentration of  $\text{CaCl}_2$  up to 0.17 molar. On the contrary, the frequency at 0.005 molar concentration of  $\text{CaCl}_2$  shows a maximal variation, 198 per cent of the normal frequency, and continuously decreases with increase in amount of the salt. The extremely low concentrations of  $\text{CaCl}_2$  less than 0.005 molar (concentration of  $\text{CaCl}_2$  in normal VAN'T HOFF solution is 0.011 molar), cause again a marked decrease in the frequency. As shown in Figs. 8 and 9, the response of heart in *O. circumpecta* to the complete lack of  $\text{CaCl}_2$  in modified BERGER solution is quite different in the frequency variation from that in *O. virginica* to the complete or extreme deficiency of  $\text{CaCl}_2$  in the VAN'T HOFF solution. This may be not only due to the difference in the proportion in concentration of other salts, but also due to the specific difference in relation of heart between these oysters. It is because, for instance, 0.418 molar solution of NaCl resumes the heart beating of *O. virginica*, while, according to TAKATSUKI (1927), the pure solution of NaCl, the concentration of which is made up equivalent to that in sea water (ca. 0.4 molar), exerts little or no effect on the heart of *O. circumpecta*.

#### SUMMARY

1. Variations in the fertilization percentage of eggs, and the amplitude and frequency of heart pulsation in oysters, *Ostrea circumpecta* and *Ostrea rivularis*, are observed in various dilutions of sea water and in different concentrations of KCl,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$  in modified BERGER solutions.
2. The fertilization percentage of eggs is not appreciably affected by such dilutions of sea water, the specific gravity of which ranges from 1.020 to 1.014 or to 1.011, but it decreases directly with further decrease

in salinity. Both the lower limit of optimum specific gravity and the minimal specific gravity for the fertilization of eggs are more or less lower in *O. rivularis* than *O. circumpecta*.

3. Generally speaking, in diluted sea water, the amplitude height varies with the variation in fertilization percentage, but the variation in the frequency is irregular: it increases to some extent with the decrease in salinity, and it decreases in further decrease in salinity.

4. Complete deficiency of any one of the three salts, KCl,  $MgCl_2$ ,  $CaCl_2$  in modified BERGER solutions, causes, in general, a decrease in the amplitude of heart beat, while it results in increase in the frequency. The grades of these variations are different in different species and in different salts.

5. Effects of the excess of the salts in the solutions upon fertilization percentage are not always in accordance with the effects of these salts on the heart pulsation. The chief differences are first the increase of the salts results generally in decrease in the fertilization percentage, and second the excess of  $MgCl_2$  and  $CaCl_2$  in the solutions causes increase in the amplitude of heart beat up to certain amounts, and only much larger excess of these salts causes decrease in the amplitude.

6. In *O. circumpecta*, the excess of KCl in the solution causes decrease in the fertilization percentage of eggs, and in the amplitude and frequency of heart beating. The variations in the first two run parallel with each other, but those in the frequency are somewhat irregular. In *O. rivularis*, the situation is quite different from *O. circumpecta*. The fertilization percentage and the amplitude of heart beat in *O. rivularis* show maximal values at certain concentrations of KCl, and both increase and decrease in such concentrations cause continuous decrease in them, but the variation in the former is very slight as compared with marked variation in the latter; and the frequency of heart beat gradually decreases almost directly with increase of the salt. Therefore, in *O. rivularis* there seems to be no intimate correlation among them at all.

7. In *O. circumpecta*, the excess of  $MgCl_2$  and  $CaCl_2$  in the solutions causes decrease in the fertilization percentage and in the frequency of heart beating, but the amplitude of heart beat increases as the concentration of the salts increases, and reaches maximum. Then it decreases with further increase in amount of the salts.

8. Thus, the fertilization percentage appears to vary, being closely related to the variation in the amplitude in some solutions, and to the variation in the frequency in the others. Sometime, it varies most closely



to the variation in the product of amplitude and frequency. However, if regardless of minute differences and roughly speaking, the relation of the fertilization percentage of eggs with the dilutions of sea water and with the concentrations of  $KCl$ ,  $MgCl_2$  and  $CaCl_2$  in modified BERGER solutions may be said to correspond generally well with that of the variation in the product of amplitude and frequency, i. e., approximately the variation in external work done per unit time by heart muscle as a whole.

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POSTSCRIPT—After receiving the print of the Fig. 7, I found my misdrawings in the marks of fertilization (solid circle) and of amplitude (circular outline) and also of frequency (cross) at 10% solution of  $MgCl_2$ , which must be placed at the height of 99.3, 94.9 and 149.5 of the ordinate respectively.

# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## X. SOME MORPHOLOGICAL AND HISTOLOGICAL PROPERTIES OF THE GRANULOCYTES OF VARIOUS INVERTEBRATES<sup>1)</sup>

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(With nine figures)

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The granulocytes are nearly constant elements in the coelomic fluid or blood of the metazoa. This type of cell has drawn the attention of many investigators and there is to be found a good deal of literature concerning the granulocytes. However, the knowledge on the nature and function of these cells is still very unsatisfactory, and this fact is particularly remarkable in the case of invertebrates. It is supposed by many authors that there is, perhaps, some relation between the mitochondria and the granules of leucocytes. The recent investigations on mitochondria revealed many new facts regarding the chemical nature of this substance. It is interesting, therefore, to reexamine the granules of the leucocytes using the modern techniques of mitochondrial investigation. This is an essential motive for undertaking the present investigation in which I also made some observations of the usual haematological properties of the granulocytes with a hope of making more or less clear the phylogenetic significance of these cells.

### MATERIALS

Materials used in this investigation are as follows:

#### Echinodermata

1. Asteroidea: *Henrica leviuscula* var. *nipponica* UCHIDA, *Aphelasteria japonica* (BELL), *Asterina pectinifera* (MÜLLER et TROSCHEL).
2. Holothuroidea: *Leptosynapta in haerens* (O. F. MÜLLER), *Caudina chilensis* (J. MÜLLER).

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## Annelida

## 3. Chaetopoda :

a) Polychaeta ; *Euphrosyne superba* MARENZELLER, *Ceratocephale osawai* IZUKA, *Nereis mictodonta* MARENZELLER, *Glycera chirori* IZUKA\*, *Travisia japonica* FUJIWARA\*, *Potamilla torelli* MALMGREN.

b) Oligochaeta ; *Pheretima sieboldi* (HORST)\*, *Ph. hilgendorfi* (MICHAELSEN)\*.

4. Sipunculoidea : *Physcosoma scolops* SELENKA et DE MAN, *Sipunculus nudus* LINNÉ, *Siphonosoma cumanense* (KEFERSTEIN).

## Molluscoidea

5. Brachiopoda : *Terebratalia coreanica* (ADAMS et REEVE), *Coptothyris grayi* (DAVIDSON).

## Mollusca

6. Gastropoda : *Cuthona* (*Cuthona*) *bicolor* BERGH.7. Lamellibranchia : *Ostrea* (*Crassostrea*) *circumpicta* PILSBRY, *Mytilus grayanus* DUNKER, *M. edulis* LINNÉ, *Anomia liskei* DAUTZENBERG et FISCHER, *Libitina japonica* (PILSBRY).8. Cephalopoda : *Idiosepius paradox* (ORTMANN), *Polypus dofleini* WÜLKER.

## Arthropoda

9. Crustacea : *Tachypleus tridentatus* LEACH, *Brachionotus sanguineus* (DE HAAN), *Huenia proteus* DE HAAN, *Eriocheir japonica* DE HAAN\*, *Spirontocaris pandaloides* (STIMPSON).

Of these animals, the species marked with an asterisk were obtained in the vicinity of Matsuyama City, the rest were collected in the neighbourhood of the Asamushi Marine Biological Station.

## METHODS

The following methods were employed in the present investigation.

1. *Staining of lipoids.* a) The specific stains for the fatty substances. Sudan III and IV (Scharlach R.), whose properties and use are well known. I employed KAWAMURA and YASAKI's method (1933) for the preparation of Sudan III solution, and HERXHEIMER's method for that of Sudan IV. In order to fix the cells, the wet cover slip preparation was exposed to the formol-vapour for three to five minutes. Then it was dipped for a

few seconds in 68 per cent alcohol.

b) Non-specific stains. i. Nile-blue sulphate. The smear fixed with 68 per cent alcohol is plunged into a saturated water solution of Nile-blue sulphate and for about 10–16 hours. Then it is differentiated with 0.1 per cent acetic acid, 2–3 minutes. The neutral fats stain reddish, fatty acids bluish with the dye. ii. Indophenol blue. The well known reagent of WINKLER and SCHULTZE for the demonstration of oxidase in tissues also shows fats which are supravitaly stained. According to ZWEIBAUM (1923), the reagent may be used immediately after the mixture of  $\alpha$ -naphthol and dimethylparaphenylene diamine hydrochloride is prepared ('Nadi' mixture) or later the indophenol blue has been formed by oxidation in air. In the first case oxidase are also coloured, but the granules disappear little by little while the fat reaction increases gradually. In the latter case the staining of fats is specific and takes place immediately.

c) Osmium tetroxide. The properties and use of this reagent are also well known.

If the positive result of Sudan staining had been obtained first, the application of other methods was frequently abridged.

2. *Demonstration of vitamin A.* The cells fixed with 10 per cent formol or formol-vapour for a while were dropped directly into a solution of antimony trichloride in chloroform. Instead of this reagent, trichloroacetic acid melted with a small quantity of water (about one forth) by the use of heat was also available.

3. *Demonstration of vitamin C.* I followed BOURNE's method (1936) which uses an acidulated solution of silver nitrate.

4. *Demonstration of glutathione.* The usual method using sodium nitroprusside and ammonium sulphate was employed for the demonstration of glutathione. Previous reduction made by introducing hydrogen cyanide in the preparation.

5. *Phagocytic ability.* To determine the phagocytic ability of granulocytes I employed MORI's method (1928) which uses India ink suspended in a 0.1 per cent solution of gum Arabic.

6. *Other methods of haematology* such as vital, supravital, or post-mortal staining, etc. were used if necessary. In the case of marine invertebrates, dry smears of blood could not be obtained successfully with the usual technique, owing to the high concentration of salts. Accordingly I frequently employed the fixation method of DREW (1911) for GIEMSA's staining.

## OBSERVATIONS

## 1. Asteroidea.

*Henrica leviuscula* var. *nipponica*. The granulocytes are colourless or slightly yellowish cells having a thin, clear ectoplasm and finely or coarsely granulated entoplasm. Occasional cells contain several green or brownish granules besides the usual colourless or yellowish granules. The nucleus is round, oval or flat disc in shape, and is usually single but on rare occasions there are two or three nuclei. Lobular or membranous pseudopodia are seen in vitro, but there is usually very little amoeboid movement. These cells have a tendency to agglutinate into numerous small masses, mixing with the other types of leucocytes. The granulocytes of the present animals may be distinguished into the following two subtypes.

i. Finely granular cells, 8–15  $\mu$  in diameter. The entoplasm is filled with numerous, fine (0.5–1  $\mu$  across), refractile and slightly yellowish granules. These granules are easily stained supravivally with erythrosin, but they show amphophilic property with intensive eosinophilic inclination when fixed and stained with GIEMSA's stain. The granules are stained orange red with Sudan III, yellowish orange with Sudan IV. Indophenol blue stains the granules greenish blue. The Nadi-mixture also stains the granules bluish, but the shade is pale and faint. Oxidase granules are fine, numerous and deep blue. In the test for vitamin A, there were encountered a few granules coloured in CARR-PRICE blue. Vitamin C granules are fine and numerous, and there was a tendency toward perinuclear condensation (Fig. 1, e–g). The ability of phagocytosis seems to be reciprocally proportional in the amount of the specific granules. The granulocytes filled with the specific granules so densely that the nucleus is completely hidden by them have no ingested ink-granules while similar cells in which the granules are sparsely scat-

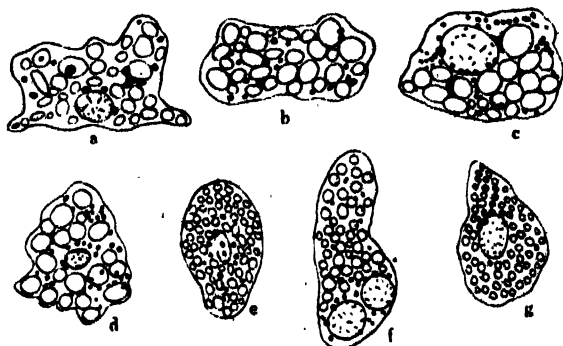


Fig. 1. Vitamin C granules in the granulocytes of *Henrica*. ca. 1,000 $\times$ .

a–d. Coarsely granular leucocytes.

e–g. Finely granular leucocytes.

tered are sparsely scat-

tered in the cytoplasm contain very numerous ink-granules. The appearance of ingested granules in the cytoplasm is also very rapid in the cells of the latter type, and it reaches the maximum ingestion within about 30 minutes. The ingested ink granules show a good deal of variation in size and shape.

ii. Coarsely granular cells,  $12-18\mu$  in diameter. These cells are infrequent and larger than the former type of cells. The granules are quite colourless, not so high refractile, and measure about  $2-3\mu$  across. These granules are amphophilic with more or less basophilic inclination. They are also sudanophilic (deep red or orange red) and less resistant to alcohol, ether, and other fat-solvents. The granules stain greenish blue with indophenol blue, and light blue with the Nadi-mixture. This type of cells is remarkably rich in vitamin C (Fig. 1, a-d), but is completely negative to ink-digestion.

*Aphelasterias japonica*. No coarsely granular cell is found in this animal. The finely granular cells are  $6-14\mu$  in diameter. Occasional cells possess several vacuoles which frequently grow to a large one by union with one another. The nature of granules, phagocytic ability, etc. are quite similar to those of corresponding cells of *Henrica*. The granules of vitamin C are rod-like in shape and ten or a dozen was the usual number present.

*Asterina pectinifera*. As to the granulocytes, only the finely granulated type is found. They are actively amoeboid, protruding lobular or root-like pseudopodia, and show most intensive ingestion of India ink in comparison with the aforementioned animals. The boundary of ecto- and entoplasm is very distinct even in the case in which the cell is at rest and has taken a spherical shape. In the entoplasm there are embedded numerous, fine, yellowish or greenish brown granules. These granules stain pink or red with MANN's stain, pale blue with the Nadi-mixture and greenish blue with indophenol blue. Two or three small granules of vitamin A are found in the perinuclear region upon very rare occasions. The granules of vitamin C are approximately half a dozen to a cell and they are aggregated usually in the vicinity of the nucleus.

## 2. Holothuroidea.

*Leptosynapta in haerens*. The colourless granulocytes are packed with numerous, fine, round granules, and measure  $10-15\mu$  in diameter. Occasional cells contain one, two or several green granules besides the usual granules. The pseudopodia are needle-like, nevertheless they were examined immediately after blood drawing. They are inactive in regard to amoeboid

movement and phagocytosis. Repeated trials failed to demonstrate vitamin A in any cell. The granules of vitamin C are approximately 6-10  $\mu$  to a cell, and there is a tendency toward perinuclear condensation,

*Caudina chilensis*. The existence of vitamin C in both the colourless and brown granulocytes was determined by the present observation. In the former, fine and very numerous granules of vitamin C were scattered throughout the cytoplasm. The brown granules of the latter blacken faintly by the reagent, but no special granules were newly produced.

### 3. Chaetopoda.

a. Polychaeta. *Euphrosyne superba*. The two types of granulocytes are distinguishable.

i. Coarsely granular cells, 8-18  $\mu$  in diameter. These cells are rather infrequent. In the entoplasm there are found numerous, colourless and coarse granules, but the excentrical and oval nucleus is not hidden with the granules. The granules are eosinophilic and stained with the Nadi-mixture and indophenol blue. They stain also orange red with Sudan dyes. The granules turned to gray or grayish green in colour with the acidulated silver nitrate solution, but no newly formed granules of vitamin C were discernible. These cells are amoeboid and phagocytic, but not intensive.

ii. Finely granular cells, 10-18  $\mu$  in diameter. Numerous, fine and bacillus-like granules are found in the cytoplasm (Fig. 2, a & b). They

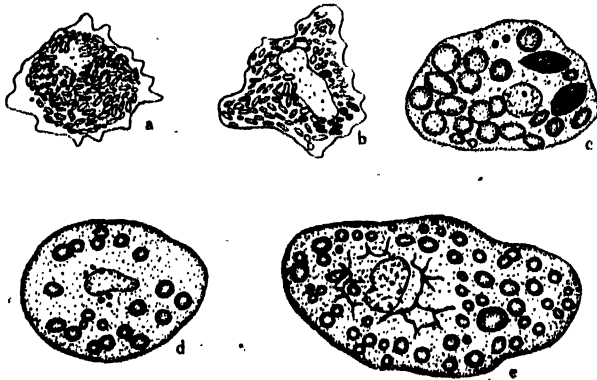


Fig. 2. a & b. Finely granular cells of *Euphrosyne* with vitamin C granules. ca. 1,000 $\times$ .

c-e. Coarsely granular cells *Ceratorcephale*. ca. 1,000 $\times$ .

c. Fresh and unstained cell with ingested spindle bodies.

d. Fresh cell with neutral red bodies.

e. Fresh cell with reticulation.

are yellowish or slightly greenish yellow in colour, and are easily disintegrated by the action of various fat-solvents. But they stain pale orange with Sudan dyes, showing a little affinity for these dyes. By the adding of Nadi-mixture to these cells the granules are dissolved by and by. Oxidase granules are fine, round, and deep blue, but not numerous. Ten or twelve is the usual number of vitamin C granules which are always in the perinuclear region (Fig. 2, a & b). In the smear preparation of blood stained with GIEMSA's stain, the granules are coloured pink or yellowish red, showing the true eosinophilic nature. These cells are amoeboid and remarkably intensive in the phagocytosis, in spite of the abundance of specific granules.

*Ceratocephale osawai*. i. Coarsely granular cells. It is well known that the largest types of amoebocytes with granules are encountered in the coelomic fluid of Annelida. The present cells are one of these types, and measure 15-30  $\mu$  in diameter. The number of granules in a cell varies strikingly; some possess only two or three granules, while the others contain several dozen of them. The size of granules in a given cell is also uneven. Besides the granules there are found several vacuoles and two or three ingested spindle bodies (Fig. 2, c) in the cytoplasm of occasional cells. The granules are colourless or slightly greenish, somewhat refractive, and show nearly no Brownian movement. They are easily soluble in alcohol, ether, and other fat solvents, and are stained deep red with Sudan III, reddish orange with Sudan IV. They are also positive to the Nadi-reaction and indophenol blue staining. It is noteworthy that some of these granules showed the positive reaction of vitamin A. A few granules of vitamin C could be seen also in the perinuclear region.

Both the cytoplasm and granules are basophilic or amphophilic with an intensive basophilic inclination in the smear fixed and stained with GIEMSA's stain. The vacuoles remain unstained by this treatment.

These cells are remarkably phagocytic, nevertheless their amoeboid movement is difficult to recognize under the microscope. The analogous appearance of these cells reminds me of the red cells of *Urechis* and *Thalassema*, of which I reported in detail in a previous paper (OHUYE, 1937 a). The fact that a structure similar to the reticulation pattern of red cells is demonstrable in the perinuclear region of these cells by using the supravital staining of Janus green B (Fig. 2, e) serves also to deepen this impression. Furthermore, the perinuclear granules similar to the 'segregation apparatus' are also stained with neutral red frequently (Fig.



2, d). I made LEPEHNE's test for haemoglobin on the present cells to ascertain whether they contain that pigment or not, but it was negative. It seems to me that these cells would be an important type to utilize in discussing the evolution of the invertebrate erythrocytes, though there is no reasonable evidence besides the facts mentioned just above. Further investigation is necessary on this point.

iii. Finely granular cells, 10–15  $\mu$  in diameter. These cells are less numerous than the former and are very similar to those of *Henrica* in appearance and nature.

*Nereis mictodonta*. The coarsely granular cells are the essential element of the coelomic corpuscles. They are hardly distinguishable from those of *Ceratocephale*.

*Travisia japonica*. Both the finely and coarsely granular leucocytes are so densely filled with the round or oval granules that the nucleus is obscured from the sight. The former are relatively numerous and small in size (8–16  $\mu$  in diameter), while the latter are less frequent and larger in size (12–25  $\mu$  across). The fine granules are stained deep orange with Sudan dyes and are amphophilic inclination to GIEMSA's staining. The coarse granules are stained orange yellow with Sudan dyes and show the amphophilic reaction with intensive eosinophilic inclination. Both type of cells are amoeboid and phagocytic. They possess a few relatively large vitamin C granules in the perinuclear region.

*Glycera chirori*. The granulocytes of these worms are quite similar to those of *Travisia* but the granules are stained more intensely (deep red) with Sudan dyes.

*Potamilla torelli*. Concerning this worm, I wish to give only a short

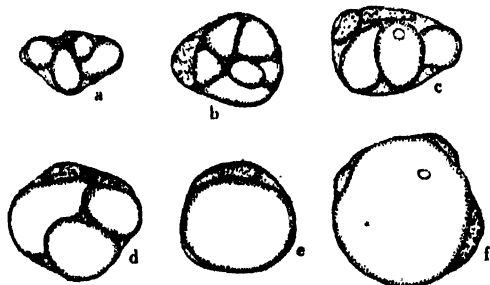


Fig. 3. Green cells of *Potamilla*. ca. 1,000 $\times$ .

account of the green cells. These cells are found in the axial lumen of branchial filaments and measure 10–25  $\mu$  in diameter. The cell body is filled with several large, green coloured granules or globules which take a morula-like arrangement (Fig. 3). It is seen frequently

that these granules gradually increase in size with the union of small granules with one another, until they form at last a large single granule which occupies almost the whole

of the cell body (Fig. 3, e & d). The cytoplasm is pressed against the cell wall, turning into the shape of a signet-ring, and usually a single, occasionally two or three nuclei are located at the thickened rim of the cytoplasm. The full grown cells are, perhaps, non-amoeboïd and non-phagocytic.

The granules are intensely stained supravitaly with neutral red, Nile-blue sulphate, brilliant cresyl blue, etc. They are stainable with Sudan dyes when the cells have been previously immersed in the isotonic solution of ammonium salts. These chemicals, as was observed by HEILBRUNN (1936) in his experiment on the eggs of *Arbacia*, act, perhaps, to loosen the protein-lipoid binding. The green granules are hardly soluble in ether, acidulated alcohol, acetone, chloroform, etc. Dilute alkali (0.02 N NaOH for example) or acid (0.02 N oxalic acid, acetic acid, etc.) have no effect on the granules, but 0.1 N NaOH dissolved them entirely. In a 0.1 N solution of HCl the green granules turn to a brownish shade. A few small granules of vitamin C are discernible in the intergranular cytoplasm. The green granules themselves become an ashy colour with the reagent of vitamin C.

b. *Oligochaeta*. *Pheretima sieboldi*. In the previous paper (OHUYE, 1937 c) I stated that the eosinophilic granulocytes of this species are slightly positive to staining with Sudan IV but negative to Sudan III. In the present investigation, it was succeeded, however, to stain also with Sudan III solution prepared by KAWAMURA and YASAKI's method (1933). The granules of lamprocytes were also positive (orange or reddish orange) to Sudan dyes. Vitamin C granules were easily demonstrable in the eosinophilic granulocytes; they are from two to several in number, relatively large in size, and localized in the intergranular cytoplasm.

*Pheretima hilgendorfi*. The result was just alike to the above.

#### 4. Sipunculoidea.

*Physcosoma scolops*. The general histology of blood corpuscles of this species was reported in my previous work (OHUYE, 1937 a). Small acidophilic granulocytes are most actively amoeboid, phagocytic (Fig. 4, i-k) and rich in vitamin C granules which are scattered throughout the cytoplasm. Middle-sized acidophilic granulocytes with spherical granules are next most active in these function (Fig. 4, l-n). Vitamin C granules amount to one or two dozens and are distributed throughout the cell body. Middle-sized granulocytes with rod-like granules are active in amoeboid movement, but inactive with respect to phagocytosis. The ingested ink-granules are about half a dozen in number and located usually

in the vicinity of the nucleus (Fig. 4. a-h). In most of these cells approximately half a dozen or more granules of vitamin C could be observed.

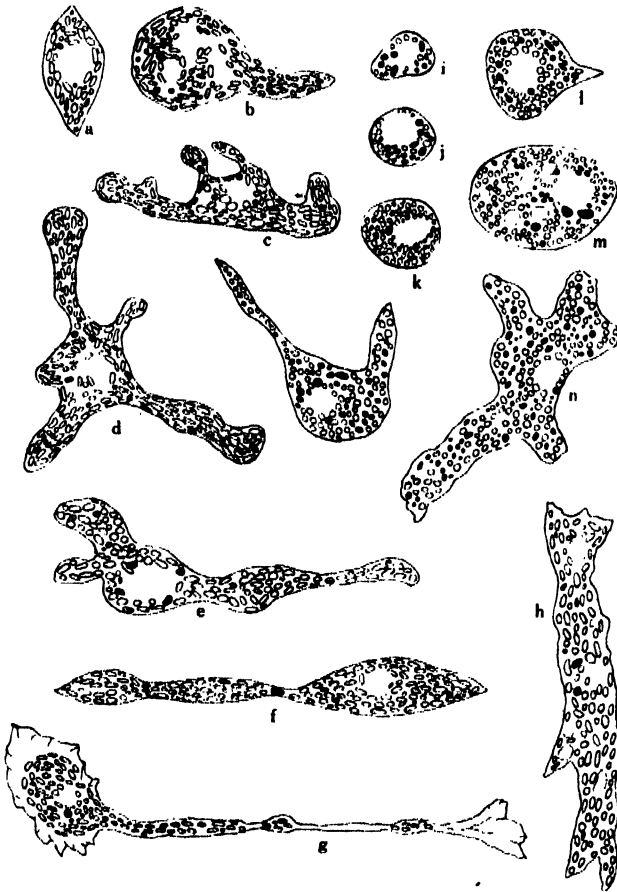


Fig. 4. Ink-ingestion of granulocytes of *Physcosoma*. ca. 1,000 $\times$ .

They were usually rod-like in shape and show a tendency toward perinuclear condensation. In the majority of the cases the specific granules of these cells were truly eosinophilic but occasional cells showed a tendency toward amphophily. Large acidophilic granulocytes were absolutely negative to ink-ingestion. Small and round granules of vitamin C were distributed in the intergranular cytoplasm. Basophilic granulocytes were a very infrequent element, and it was difficult to distinguish them from the acidophilic granulocytes in the unstained preparation. I failed to demonstrate vitamin C in the cells of urns.

I made observations on the granulocytes of *Sipunculus nudus* and *Siphonosoma cumanense*, and a similar result to that of *Physcosoma* was obtained.

#### 5. Brachiopoda.

*Terebratalia coreanica* and *Coptothyris grayi*. The histology of coelomic corpuscles of these brachiopods was the subject of my foregoing papers (OHUYE, 1936 a, 1937 b). It is only noteworthy here that the amoebocytes with brown granules which aggregated in the tissue of cirri contain very abundant vitamin C and glutathione in the whole cell body. By the treatment with acidulated silver nitrate the whole brown granules turned to brownish black or black showing that the granules contain vitamin C in their own structure.

As to glutathione it was supposed that this substance would be easily demonstrated in the blood cells of all animals. In reality, however, the demonstration of glutathione did not succeed in any case except with the animals under discussion. The brown granulocytes turn to a beautiful wine red colour immediately after the application of the reagent. This shade lasted for one hour or more with no remarkable fading. The reaction occurs equally even in the case which the previous reduction of material was not made. Accordingly it is evident that glutathione of the present case occurs as the reduced form.

The abundance of vitamin C and glutathione makes us to suppose that the brown granulocytes of brachiopods should possibly play a role of respiration. This view is also supported by the fact that these are usually found in the tissue of cirri from which oxygen is taken up. Similar supposition has been made by LEBLOND and his co-worker (1934). They postulated that vitamin C is associated with the respiration of the cell and therefore its abundant existence is found in the alveoli of lung where respiratory exchanges are rapid and continuous.

The amoebocytes with red granules contain also numerous vitamin C granules while the detection of glutathione was not successful.

#### 6. Gastropoda.

*Cuthona (Cuthona) bicolor*. This small nudibranch possesses branchial papillae arranged on each side of the back. Numerous green cells are found in the axial lumen of these branchial papillae. Such structure reminds us of the branchial filaments of *Potamilla*. The green cells are filled with granules which are nearly uniform in size in a given cell but show a remarkable variation such as  $0.5\text{--}3.0\ \mu$  across among individual cells. The colour of the cells is greenish blue in the freshly captured

specimens, but it fades gradually and turns to an olive yellow within two or three weeks after the captivation. The green granules have intensive affinity for the various dyes such as neutral red, Nile-blue sulphate, brilliant cresyl blue, methylene blue, etc. which were applied supravitaly. In the smear stained by GIEMSA's method the cytoplasm stains purplish blue and the granules deep greenish blue, showing their basophilic property. The staining with Sudan dyes is usually negative, but the previous treatment with ammonium salts makes it possible to stain the granules in the colour of orange red, as was true in the case of *Potmilla*. By close examination it will be seen that the granules are composed of two parts; the central and the cortical portions. The former is nearly colourless or light greenish, and stained deep red with neutral red, Sudan III, etc. The latter is a green sheath and is difficult to stain with these dyes. From these facts it may be understood that the granules consist of the lipoidal central mass and pigmented cortical layer. The Nadi-mixture and indophenol blue stain the granules greenish blue or blue. In this case, however, it is difficult to distinguish the cortical and medullary portions, because both the portions are stained in a similar hue. With the acidulated silver nitrate solution all green granules turn into slate-green or dark ashy shade, showing the reduction of silver nitrate (Fig. 5). Among these specific granules there are found numerous deep brownish or black granules which are somewhat smaller than the

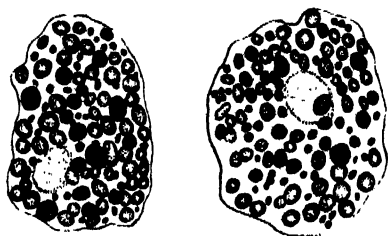


Fig. 5. Green cells of *Cuthona*, treated with an acidulated solution of silver nitrate. Vitamin C granules are black; specific granules are spotted in proportion to the intensity of silver-nitrate reduction. ca. 1,000 $\times$ .

specific granules. From these facts it is understood that the green cells are very rich in vitamin C.

The green colour of the granules is faded by the action of various acids such as 0.1 N solution of HCl, H<sub>2</sub>SO<sub>4</sub>, etc. 0.1 N solution of NaOH dissolves the granules. In acidulated alcohol they are dissolved or perfectly discoloured. The granules are slightly soluble in ether and chloroform, but insoluble in acetone, toluol, benzol, xylol, etc.

The green cells are able to change their shape very slowly, but are not phagocytic.

The colourless granulocytes found in the coelomic fluid are basophilic,

sudanophilic and positive to the reaction of vitamin C.

#### 7. Lamellibranchia.

*Ostrea* (*Crassostrea*) *circumpicta*. TAKATSUKI (1934) published an elaborate work on the nature and function of the amoebocytes of the European oyster, *Ostrea edulis*. The results of the present observation are similar to his except in some details. As was described by TAKATSUKI, the granulocytes of the oyster are very active in amoeboid movement and phagocytosis. It seems to me that concerning these properties the granulocytes of the present species are the most active among 30 species examined in this investigation. They continued amoeboid movement thrusting out blunt lobular pseudopodia for several hours. They ingested the India ink so intensely that their cell body was packed with the ink granules, which are somewhat larger than the specific granules, within about 20 minutes.

The granules are stained intensely and quickly with brilliant cresyl blue, Nile-blue sulphate, neutral red, etc. Erythrosin stains the cytoplasm but never the granules. The granules were stained in orange red or deep red with Sudan dyes, while TAKATSUKI has reported a negative result on this point. Some of granules grew somewhat larger than the normal size through this staining, presumably due to the union of several granules with each other. This change was less remarkable in the staining with Sudan IV than with that of Sudan III. The Nadi-reaction occurs only in the surface layer of granules, and, accordingly, the granules showed various shades such as greenish lavender, lilac or aconite violet, etc., accompanying the change of the focussing depth in the microscopic examination. Vitamin C granules are approximately half a dozen in number and show a remarkable inclination toward perinuclear condensation. In the smears of blood stained with GIEMSA's dye the cytoplasm of the granulocytes stains eosinophilic or amphophilic with an inclination of eosinophily while the granules are truly and non-metachromatically basophilic. The contour of stained granules is very distinct.

TAKATSUKI described the changes of the amoebocytes in the hypotonic solution in detail. I also made an observation on the effect of slightly hypotonic sea water (sea water was diluted with one third bulk of distilled water) on the granulocytes of the present specimens. The result was, of course, essentially similar to that of TAKATSUKI. Here I will describe only the interesting behaviour of granules which was found by the present observation. By the addition of the dilute sea water, the granules accumulate soon or later at the centre of an expanded and spherical cell

(Fig. 6, b), and show most active Brownian movement at this time; then the granules located at the margin of the accumulated mass become less

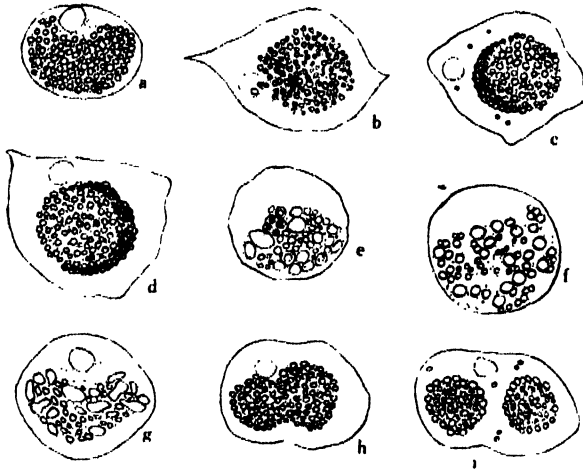


Fig. 6. Effect of hypotonic sea water on granulocytes of oyster. ca. 1,000 $\times$ .

a. Cell in the normal sea water.

b-i. Cells in a hypotonic sea water.

active in movement, and grow into larger ones by the mutual fusion of several adjoining granules. In these granules the Brownian movement stops perfectly. Such change takes place not on all the surface of the mass of aggregated granules but only at an optional point. This change gradually progresses from the initial place in all directions, in the shape of a crescent (Fig. 6, c & d) firstly, then of

a signet-ring, and, at last, of a ring with a rim of uniform thickness. In this stage the Brownian movement of all granules found in the cell stops completely, and the mutual fusion of granules occurs not only on the surface, but also in the deep part of granular accumulation (Fig. 6, e). There are occasionally found two granular accumulation in a cell (Fig. 6, i). As time goes by, the accumulated granules begin to scatter throughout the cytoplasm, and the cell body is occupied again by the granules of irregular size and shape (Fig. 6, f & g).

*Mytilus grayanus* and *M. edulis*. The granules of these animals are very similar in every way to those of the oyster.

*Libitina japonica*. Finely granular amoebocytes occur abundantly in the body fluid and are also essentially analogous to those of the oyster. The coarsely granular leucocytes, measuring 12–20  $\mu$  across, contain from three to thirty or more granules in the cytoplasm. They measure about 2–3  $\mu$  in diameter, and some of them possess yellow or brown pigment. The granules are sudanophilic and amphophilic with an intensive eosinophilic inclination. These cells are inactively amoeboid and phagocytic. Vitamin A test was negative. Vitamin C granules are few, small and perinuclear.

*Anomia liscki*. The granulocytes which contain fine, numerous and slightly yellowish granules were acidophilic while those of the four species mentioned just above had been basophilic.

#### 8. Cephalopoda.

*Idiosepius paradoxa*. There are seen a good many of granulocytes which contain numerous, fine, refractive and colourless or slightly yellowish granules in the blood of this species. They measure 8–15  $\mu$  in diameter and are remarkably amoeboid and phagocytic. The granules are positive

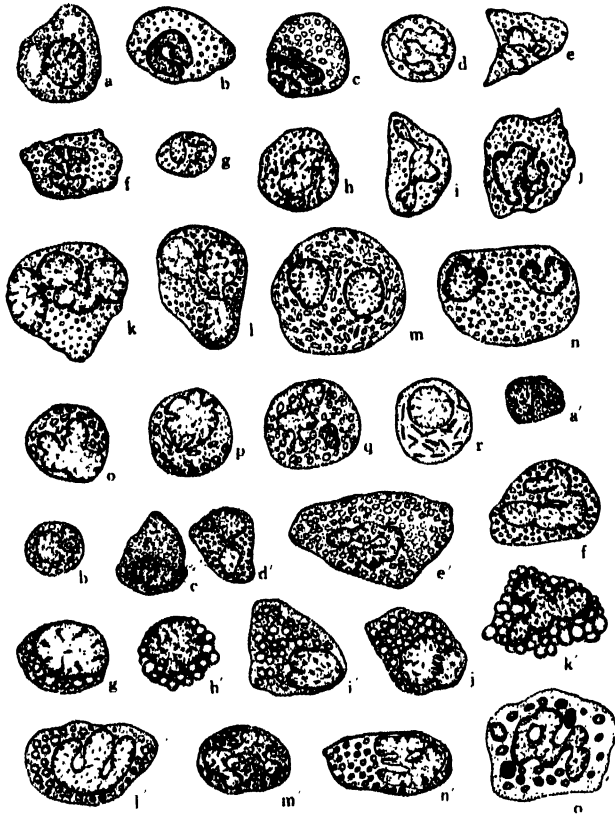


Fig. 7. Granulocytes of cephalopods. ca. 1,000 $\times$ .

a-n. Eosinophilic, o-r. Basophilic granulocytes of *Idiosepius*.  
a'-f'. Eosinophilic, g'-o'. Basophilic granulocytes of *Polypus*.

to the Nadi-reaction and the supravital staining with indophenol blue, Nile-blue sulphate, neutral red, etc. Vitamin C granules are round, perinuclear, and approximately half a dozen in number. In the smears of



blood stained with GIEMSA's dye, the granulocytes are eosinophilic or amphophilic with an eosinophilic inclination (Fig. 7, a-n), and basophilic or amphophilic with basophilic inclination (Fig. 7, o-p). Occasional cells possess simultaneously both kinds of granules (Fig. 7, q); namely basophilic coarse and eosinophilic fine granules. Cells with numerous basophilic rodlets were met with occasionally too (Fig. 7, r).

As to the nucleus of Cephalopoda, CUÉNOT (1891) described it as follows; "il (le noyau) est tentot profondément bilobé ou trilobé, tantot contourné. . . ; le noyau des cellules jeunes, pleines de granules albuminogènes, est régulièrement sphérique ou ovoïde, et c'est seulement en avançant en âge qu'il se déforme ainsi." KİYONO (1929) stated also the presence of the horseshoe-shaped or bilobular nucleus in the blood of an octopod. In the present species also the nucleus shows a good deal of variation in its shape. It is usually round, oval or bean-like in shape, but occasional cells possess bi- or tri-lobular polymorphic nucleus similar to that of neutrophilic granulocytes of the vertebrates. Such a polymorphic nucleus is found also in the hyaline amoebocytes. The amitoses of nuclei of circulating blood cells are encountered occasionally. It is accepted generally that there is no polymorphic nucleus in the blood corpuscles of the invertebrates. This idea, however, seems to be incorrect so far as it concerns to the blood of *Cephalododa*.

*Polypus doeleini*. There is no fundamental difference between the granulocytes of the present species (Fig. 7, a'-o') and those of *Idiosepius*.

#### 9. Crustacea.

*Tachypleus tridentatus*. This species was classified as a crustacean for the sake of convenience. Regarding the nature, especially the coagulum formation of blood corpuscles of *Limulus polyhemus*, there are many works published by ALSBERG and CLARK (1908), LÖEB (1910), etc. In the present paper the chief interest was to study the staining reactions and lipoidal nature of the granulocytes.

The granulocytes are actively amoeboid cells (Fig. 8), measuring approximately 10-20  $\mu$  in diameter, but the phagocytosis is not remarkable in vitro. The entoplasm of full grown cells is usually packed with both fine and coarse granules in various proportions (Fig. 8, d-i). The former are round, colourless and nearly uniform in size in a given cell. The latter are round, oval or polygonal in shape, slightly yellowish or greenish yellow in colour, and show a good deal of variation in size. These differences of the two granules, however, are not distinct in the young cells.

Vacuoles are frequently found in the cytoplasm in various number and sizes (Fig. 8, e-g).

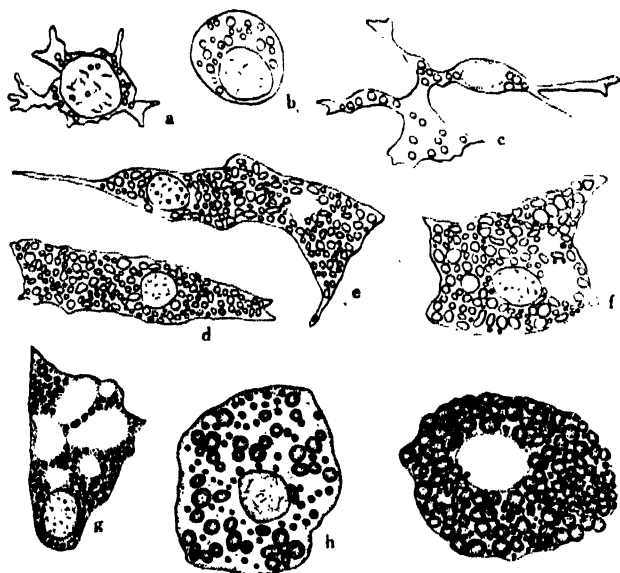


Fig. 8. Granulocytes of *Tachypleus*. ca. 1,000 $\times$ .

a-f. Fresh and unstained cells.

g-i. Supravitally stained with brilliant cresyl blue.

Since a large amount of blood is obtained easily from this animal, I made a rather detailed observation of the granulocytes, which is described as follows.

1) Vital staining. The dyes injected intra vitam are brilliant cresyl blue, carmine, methylene blue, neutral red, Nile-blue sulphate and trypan blue. Two full grown animals received four injections of 1 c.c. of 0.1 per cent solution of a dye intraperitoneally every other day. In total, twelve animals were used for the purpose of the vital staining. Two days after the last injection the blood was drawn and examined. The vital granules of brilliant cresyl blue and Nile-blue sulphate are few, round and deep blue, and occur usually in the vicinity of the nucleus. Those of neutral red are relatively numerous, irregular in size, and eosin pink in colour. Trypan blue and carmine were not ingested in the granulocytes while they were occasionally found in the cytoplasm of hyaline leucocytes.

2) Supravital staining. The results are seen in Table 1. In the table the staining reaction of both kinds of granules was only given.

TABLE 1.

*Supravital staining of the specific granules in the granulocytes of Tachypleus tridentatus.*

Dyes	Fine granules	Coarse granules
Brilliant cresyl blue	blue	sea green or Venice green
Carmin (lithium)	unstained	unstained
Cresyl violet	unstained	slightly purplish
Eosin	intensely stained in bright pink	slightly pink
Erythrosin	slightly pink or unstained	intensely deep red
Fuchsin acidic	slightly purple	unstained
Gentian violet	unstained	unstained
Janus green B	greenish blue	slightly greenish
Light red	red	red
Methyl green	unstained	unstained
Methylene blue	blue	Venice green or calamine blue
Neutral red	eosin pink	slightly pink
Nigrosin	unstained	unstained
Nile-blue sulphate	deep blue	Venice green or peacock blue
Orange G	slightly yellow	slightly yellow or unstained
Safranin O	unstained	unstained
Trypan blue	deep blue	blue or greenish blue

It is seen in Table 1 that the dyes which are easily soluble in the lipoids, such as brilliant cresyl blue, Nile-blue sulphate, etc. show the intensive staining of the granules. It is also seen in this table that the granules are stainable both basic and acidic dyes. This is a property of acidophilic leucocytes.

3) Staining after fixation. In the smears stained by GIEMSA's method, the fine granules are stained pink or eosin pink while the coarse granules are unstained or slightly stained in a similar manner. Triacids solution stains the former violet red and the latter bright copper red. These staining reactions show that these granulocytes are acidophilic.

4) Staining of fatty substances. The fine granules are stained orange red with Sudan III. The coarse granules are also stained in a similar manner, but they are frequently dissolved by the staining. Sudan IV stains the fine granules peach-red or red orange, and the coarse granules orange or orange-red. Osmic acid (2 per cent) causes the colouration of the granules; the fine granules turn brownish yellow and the coarse granules brown or Sudan brown.

5) Action of various fat-solvents. The results are seen in Table 2.

TABLE 2.

*Solubility of the granules in the granulocytes of Tachypleus in various solvents.*

Solvents	Fine granule	Coarse granules
Aceton	insoluble	soluble
Alcohol ethyl	soluble	easily soluble
Alcohol methyl	insoluble	hardly soluble
Benzol	soluble	soluble
Carbon bisulphide	soluble	soluble
Chloroform	soluble	soluble
Ether	soluble	easily soluble

The solubility of both granules in various solvents shows nearly a parallelism except regarding aceton. KUTSCHARA-AICHENBERG (1925) stated that unsaturated or saturated phosphatids and cerebrosids are insoluble in aceton while glycerin ester, cholesterin ester, some disintegrated lipoidal substances and aceton soluble phosphatids are dissolved in this solvent. According to this description it is supposed that the fine granules would be composed essentially of phosphatids or cerebroside. Furthermore that the granules are soluble in ethyl alcohol and others, and insoluble in methyl alcohol makes us suppose that the granular substance would be unsaturated phosphatids such as lecithins. Similarly it is supposed that the coarse granules would have contained neutral fats or cholesterin esters. I mentioned just above the primary 'blackening' of granules due to the action of osmic acid. According to MURON (1904) this is only a specific reagent for unsaturated fatty acids. MURON has proved that the series of saturated fatty acids could not be blackened by osmic acid, but a trace

of impurity, that is to say, of oleic acid, is sufficient to change a negative reaction to a positive one. He stated also that when the amount of oleic acid is less than 50 per cent the colour is never black, but brown, gray or yellow. In accordance with this description it is also supposed from the results of the present investigation that both granules contain oleic acid whose amount is far less than 50 per cent, and that the amount of oleic acid is more or less larger in the coarse granules than in the fine ones, judging from the degree of colour change.

6) Reaction of proteins. Biuret, MILLON's and ninyhydrin reaction were entirely negative. But trypsin digested the granules within about three hours.

7) Reactions of carbohydrates. In the cytoplasm there are a few granules which become brownish in iodine vapour, and are stained slightly with BEST's carmine. The iodine test was first applied by EHRLICH to air dried films of human blood, and in this state there was a diffuse browning of the neutrophiles' protoplasm. NEUKIRCH (1910), using BEST's carmine, came to the conclusion that the material is either glycogen in loose combination within the cell or allied insoluble form of carbohydrate. This conclusion may hold true in the present case too.

8) Ferments. The Nadi-mixture stains the fine granules deep blue and the coarse granules slightly indigo blue or greenish blue which will fade soon later. I failed to detect peroxidase, tyrosinase, or Dopa-ferment in the granulocytes.

9) Vitamins and glutathione. Vitamin A is demonstrated by the CARR-PRICE method in the coarse granules upon very rare occasions. Vitamin C granules are fine and numerous and are found mixed with the specific granules. The reaction of glutathione was negative.

*Brachionotus sanguineus*. Most of the granulocytes of this species are 10-15  $\mu$  across and contain numerous round granules which would be difficult to find. The coarse granules are easily soluble in absolute alcohol but hardly soluble in ether and acetone. Sudan III stains them reddish yellow and Sudan IV orange or orange red. They are easily blackened by osmic acid. In the smears stained with GIEMSA's dye the granules are amphophilic with intensive eosinophilic inclination. The nucleus is large, round or oval in shape, and rich in fine chromatin granules. Vitamin C granules are few and infrequent.

The blood cells of two other crabs (*Huena proteus* and *Eriocheir japonica*) are essentially similar to those of this species, except that the granulocytes of fresh water crab, *Eriocheir*, are basophilic or amphiphilic

with intense basophilic inclination.

*Spirontocaris pandaloides*. In the blood of this green shrimp there is encountered frequently a kind of granulocytes which measure 10–18  $\mu$  in diameter and contain very numerous fine and quivering moving granules (Fig. 9, a-c). They are actively amoeboid and phagocytic. The granules show also sudanophilic and eosinophilic tendency, and are positive to the Nadi-reaction. Vitamin C granules are relatively numerous, round or rodlet, and occur throughout the cytoplasm or show a tendency toward perinuclear condensation (Fig. 9, e-j). The nucleus is frequently very voluminous and rich in chromatin granules (Fig. 9; b, c, etc.). The coarse granular cells which are found very rarely show also the amphophilic reaction with slightly eosinophilic inclination (Fig. 9; d).

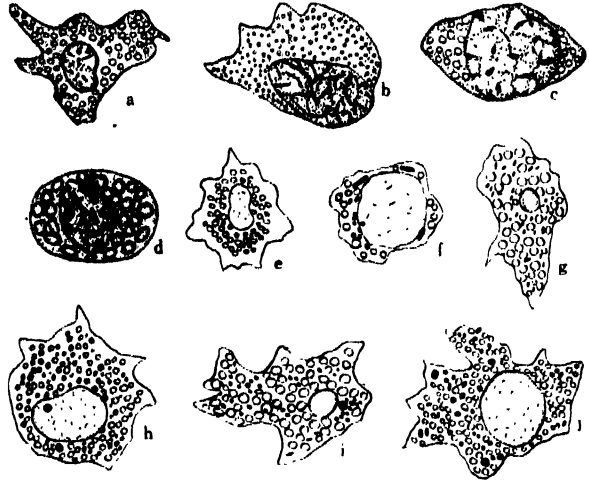


Fig. 9. Granulocytes of *Spirontocaris*. ca. 1,000 $\times$ .  
a-d. Granulocytes fixed and stained by GIEMSA's method.  
e-j. Granulocytes with vitamin C granules.

#### COMPARISON AND DISCUSSION

In the animals used for the present investigation, at least four kinds of granulocytes are distinguishable; A) granulocytes with fine granules, B) granulocytes with coarse granules, C) granulocytes with both kinds of granules, and D) pigmented granulocytes. Animals with granulocytes of types A and B simultaneously are most common; the next are those which possess cells of type A only; animals which possess cells of type B only or animals with pigmented granulocytes are encountered infrequently. The granulocytes of type C are usually very large in size, and they measure about 30  $\mu$  or more in diameter in the extreme case (Sipunculoidea).

Most of the granulocytes are amphophilic with acidophilic inclination. Amphophilic granulocytes with basophilic inclination are next most abundant.

Real acidophilic or basophilic granulocytes are encountered rather infrequently. Leucocytes are found with both kinds of granules (amphophilic ones with basophilic inclination and those of acidophilic inclination) simultaneously in the blood of the cephalopods. In the higher vertebrates, needless to say, the amphophilic granulation is found only in young forms, never in adult forms. Accordingly it is an evidence of low differentiation of leucocytes that the amphophilic granulation is rather common in the blood of invertebrates.

KOLLMANN (1908) made a study in respect to the affinity for dyes of the granulocytes in a number of invertebrates. He divided the properties of the granulocytes of Lamellibranchia into two groups according to their habitat; those of fresh water molluscs are amphophilic with a tendency to become stained by acidic dyes, and those of marine molluscs are acidophilic. If this is true the granules of the amoebocytes of Lamellibranchia which I examined should be acidophilic. But the results obtained by the present investigation were against this principle except in the case of *Anomia*, and the granulocytes of the rest showed typical basophilic reaction. TAKATSUKI (1934) reported also that the amoebocytes of the European oyster do not show the acidophilic reaction but the neutrophilic.

Now I wish to consider the polymorphic nucleus found in the blood cells of Cephalopoda. As to the blood of vertebrate animals, the significance, classification, etc. of the polymorphic nucleus were discussed in detail by ARNETH (1904, '07, '20, '25, '26). He made most striking contributions concerning the alternation of nuclear lobation in circulating blood (ARNETH index). COOKE (1914), COOKE and PONDER (1927), SCHILLING (1926), and others modified ARNETH's count of the polymorphnuclear leucocytes in a manner making both for simplicity and accuracy. COOKE stated that "If there is any band of nuclear material except the chromatin filament connecting the different parts of a nucleus, that nucleus cannot, for the purpose of count, be said to be divided." According to this statement there is no polymorphic nucleus in the blood of *Polypus dofeini*, but in the blood cells of *Idiosepius paradoxa* upon rare occasions. Namely some nuclei of leucocytes consisted of two or three knob-like lobes connected with fine chromatin filament. Most of the nuclei found in the cephalopod blood-cells should be recognized as the nucleus of M-type (round or oval), W-type (slightly indented), T-type (deeply indented), 2K-type (nucleus with two knob-like lobes), or 2S-type (nucleus with two S-like lobes) of ARNETH classification.

Recently SUGIYAMA and his co-workers made extensive investigations

on the correlation between nuclear lobations and functions of leucocytes. ONODA (1934), working under SUGIYAMA's guidance, found that there is positive correlation ( $r = +0.14191 \pm 0.0504$ ) between the wandering velocity and the average number of nuclear lobations of vertebrate leucocytes (neutrophiles, pseudoeosinophiles, amphophiles, etc.). SUGIYAMA (according to his kind verbal communication) explained this fact that the increase of nuclear lobations is an adaptation so as to easily pass the intercellular spaces of blood capillaries. Therefore it seems to be reasonable, according to SUGIYAMA, that the real nuclear polymorphism is found only in the vertebrates which possess closed and highly differentiated vascular systems. That the polymorphic nucleus was found in the blood cells of invertebrates raises a new question.

Throughout the kinds of granulocytes described in the present paper, the coarsely granulated ones when examined under the microscope, are negative regarding phagocytosis, while the finely granular ones are more or less actively phagocytic. This recalls to me the relation between the neutrophiles and eosinophiles or basophiles of the vertebrates; i. e., the former are finely granulated and show active phagocytosis while the latter are filled with the relatively coarse granules and their phagocytic ability is either very slight or uncertain. Correlation between the chromatic affinity and phagocytic ability of the leucocytes was not discernible.

A centre of the clinical haematology was to study whether the normal and healthy granulocytes contain fatty substance or not. Our knowledge on this problem has advanced with long strides within the last score of years, and there is an extensive literature dealing with this problem. According to SEHRT (1927) the exact demonstration of lipoids in the normal and healthy leucocytes was first made by SAVINI (1921). He succeeded in staining the granules of human eosinophiles, neutrophiles and mast cells a red colour with Sudan IV, after the fixation with copper bichromate. He studied also the solubility of granules in various fat-solvents, and came to a conclusion that the various granules of leucocytes might be classed as lipoids. NEUMANN (1926) also found that the  $\alpha$ -granulation of eosinophiles in horse blood normally contains a lipoidal substance which constitutes the characteristic structure of these granules. In this wise the lipoidal nature of the leucocytic granules has long since been determined in mammals. In the survey of literature treating invertebrate haematology, however, it is seen that experiment usually failed to demonstrate the presence of lipoids in the granulocytes. In my previous papers (OHUYE, 1936 c, d; 1937 a) I also reported frequently negative



results regarding the Sudan-dye staining of leucocyte-granules. The cause of failure of fat-staining was perhaps due to the unsuitable fixation of cells and the quality of Sudan dyes. In the case of marine invertebrates, dry smears of the blood and body fluid cannot be successfully obtained in the majority of cases, as the quantity of salts in solution in the blood, etc. prevents the almost instantaneous drying which is necessary, and also gives rise to the formation of crystals which causes much distortion of the organized elements of the blood. Accordingly it is necessary to fix the film of blood while wet. The following treatment of preparation with 68 per cent alcohol after fixation prevents the corpuscles from becoming detached from the glass during the staining. That the quality of Sudan III is not constant even in the preparations of GRÜBLER & Co., was pointed out by SEHRT (1927). In fact, one preparation of dye stains a lipid well while another does not give the same stain. It is necessary, therefore, to examine the quality of dye previous to its use. Sudan IV is, needless to say, a diazo-naphthalene compound similar to Sudan III except that it is a dimethyl derivative. This fact makes it a deeper, more intense stain; therefore, it is one of the best fat stains known at present. As is seen in the foregoing descriptions, the granules of leucocytes show a positive reaction almost constantly to the staining with Sudan dyes, although the shade of stained granules varies from pale orange to deep red. That Sudan dyes are able to stain not only fats and soaps, but various fatty acids and lipoids also became clear recently. PARAT (1927) listed the names of lipoids stainable with Sudan dyes, in the order of staining intensity; i. e., neutral fats, fatty acids, soaps, mixtures of fatty acids and cholesterol esters, mixtures of neutral fats and cholesterol esters, phosphatids, cerebrosids and certain lecithins. According to him the first three are, of course, stained red, the next two red or yellowish red, and the last three reddish yellow or yellowish. The results of the present observation showed also such variation of shade, as was mentioned above. This shows, I think, the probable existence of substances corresponding to the shades.

The primary blackening of the granules due to the action of osmic acid was not seen in the majority of cases. This may be recognized as evidence of the total absence of unsaturated fatty acids in usual granules.

Most of the granules showed positive reactions to the staining with indophenol blue and the Nadi-mixture. It is unnecessary to state that the former is one of fat staining. As to the latter there is much dispute whether this reagent is specific to stain only the oxidase in the

cells or to stain simply fats. I believe, however, that Nadi-mixture stains truly the oxidase, as the granules were stained bluish before the indophenol blue was synthesized in the medium. This fact should be recognized as a datum which confirms NEUMANN's view on the nature of oxidase in the leucocyte granules of invertebrates.

The granules stained with the Nadi-mixture are somewhat more infrequent than those stained with indophenol blue. I suppose, therefore, the reaction of oxidase would be closely related to the lipoid, but all the lipoid granules of the leucocytes do not always contain the oxidase.

The histochemical demonstration of vitamins drew attention of many recent workers. JOYET-LAVERGNE (1935) has shown by a series of cytological and chemical preparations that vitamin A and glutathione are constant constituents of mitochondria, and this was confirmed by BOURNE (1935). In mitochondria and GOLGI apparatus, BOURNE (1933, 1935) and GIROUD and LEBLOND (1934, 1935) determined also the presence of vitamin C and glutathione. The cytological demonstration of vitamins has been made in the case of nearly all kinds of animal tissues, but scanty attention has been paid to the blood corpuscles, so far as I am aware. GIROUD and his co-workers (1934) discovered that a slight but definite reaction is given by the blood cells of guinea pigs and others. ZIMMET and FERRIÈRE (1936) also obtained a similar result on the blood of toads. STEPHENS and HAWLEY (1936) reported that the ascorbic acid was present in much greater concentration in the human leucocytes than in the erythrocytes or plasmas, and the high values were obtained in the blood of leukaemic patient. As to the invertebrates, recently the presence of vitamin C was reported by GIROUD and RATSIMAMANGA (in 12 species of animals belonging to Annelida, Echinodermata, Mollusca and Arthropoda; 1935), and v. LUDANY (in one fresh crab; 1936) but no description on this substance in blood corpuscles was given. I have reported in a previous paper (OHUYE, 1937 a) the existence of vitamin A in the blood cells of *Urechis* and *Thalassoma*. In the present investigation, as was mentioned above, this substance was found in the granulocytes of some Polychaeta, Asteroidea and Crustacea infrequently. I suppose that the difficulty of demonstrating vitamin A in the blood cell is due, perhaps, to the inappropriateness of the CARR and PRICE reaction when applied to cytological purpose. I will save the discussion of this problem for another occasion.

Whether a substance found in the cells which is able to reduce acidulated silver nitrate would be ascorbic acid or not is yet problematic (KING, 1936). But I called it vitamin C, following the view of BOURNE

and the workers of the Paris school. As was mentioned before, vitamin C was found in practically all granulocytes. In respect to this substance they should be divided into the five following groups:

- A. Preexistent specific granules giving no reaction of vitamin C.
  - 1. Vitamin C granules appear newly throughout the cytoplasm.
  - 2. Vitamin C granules show more or less a tendency toward perinuclear condensation.
  - 3. Vitamin C granules occurring only in the perinuclear region.
- B. Preexistent specific granules showing the reaction of vitamin C.
  - 4. Newly formed vitamin C granules are also to be seen in the cytoplasm.
  - 5. No newly formed vitamin C granules are found in the cytoplasm.

Most of the granulocytes belong to group A, especially groups 1 and 2. Needless to say, however, many intermediate forms were found in group A. Cells which should be classed as group 4 were infrequently encountered; the green cells of *Potamilla* and *Cuthona* belong to this category. In these cells the reduction of silver nitrate by the specific granules was not intensive. As to the fifth group one can include the brown amoebocytes of brachiopods, those of holothurids, and the coarse granules of *Euprosyne*. The most intensive reaction was seen in the first mentioned cells, and the specific granules were perfectly blackened with the reagent, showing the abundant existence of vitamin C. These cells gave also intense reaction of glutathione which I failed to detect in any cell of the other animals. The failure would be due, perhaps, to the fact that glutathione in these cells is contained in the oxidized form, and the previous reduction which I had made was unsatisfactory. Whatever be the case, it is very interesting that the specific granules of some leucocytes showed most intensive reaction of vitamin C and glutathione. BOURNE (1935) suggested that vitamin C and glutathione form an oxidation-reduction system in mitochondria. That was, I suppose, also the case in the present granules. The fact that the cells which contain abundant vitamin C, such as brown granular amoebocytes of Brachiopoda, green cells of *Potamilla*, *Cuthona*, etc. are found in the tissues of respiratory organs may suggest the existence of close association between vitamin C and the respiratory function of animals.

The vitamin C granules demonstrated in the cytoplasm by the action of the reagent were usually fine and round or rod-like ones throughout all the granulocytes. According to BOURNE (1935) these should be re-

cognized as the mitochondria. He stated: "Mitochondria are suggested as being composed of an outer lipoidal cortex which contain vitamin A and / or carotenoid pigments and core (a water-rich phase) in which vitamin C and glutathione are situated. . . ." These four substances (lipoids, vitamins A and C, and glutathione) were also found in the granulocyte. The presence of mitochondria in the human neutrophils and eosinophils was first demonstrated by COWDRY (1914). SABIN and her co-workers (1924) believed that, in the transformation of the myelocyte in the leucocyte, there is a gradual reduction in number of mitochondria as the specific granules increase in number. Analogous behaviour of vitamin C granules to this description was found in the present investigation: In the hyaline amoebocytes of various invertebrates, of which I will give account in another article, there are usually found very numerous vitamin C granules, and their appearance, size and state of aggregation are quite similar to the mitochondria which are demonstrated by the supravital staining of Janus green B. Vitamin C granules are less in number in the granulocytes than those hyaline amoebocytes, and there is a tendency for the granulocytes which contained numerous specific granules to be relatively sparse in vitamin C granules and vice versa, although there are, of course, many exceptions to such a generalized principle.

Whether the specific granules of leucocytes would be the product of mitochondrial differentiation or not is an interesting question, but not yet solved at present. Since the majority of granules did not show the vitamin C reaction, it would be supposed that they are not substance produced by the direct metamorphosis of mitochondria. Regarding the granules of brown amoebocytes of brachiopods, which show the most intense reactions of vitamin C and glutathione, there is probability of this transformation. The cells belong to the fourth group, I suppose, show intermediate characteristic between these two types. Is it too hasty to suppose that the granulations of Branchiopoda still retain the mitochondrial characters from which they were metamorphosed, while in those of usual animals so highly or specially differentiated the properties of the mother substance have been lost in the course of metamorphosis?

In conclusion, lipoids, oxidase, vitamins A and C, glutathione, etc. were found in the granulocytes of various invertebrates. It is evident that these substances would play an important role in cellular respiration. I think, therefore, the granulocytes should be reexamined with respect to the relation between the function of granulocytes and these respiratory substances.

## SUMMARY

1. Four kinds of granulocytes, at least, are found in the blood or body fluid of various invertebrates; a) finely granular cells, b) coarsely granular cells, c) cells with both fine and coarse granules, and d) pigmented granulocytes. They are basophilic, eosinophilic, or amphophilic with inclination toward either the basophilic or eosinophilic.

2. The granulocytes are amoeboid with a few exceptions. The finely granular cells usually show phagocytosis, but the others are almost negative to this function.

3. The majority of specific granules are stained with Sudan dyes, indophenol blue, and the Nadi-mixture, showing that they contain certain lipoids and oxidase. The granulocytes are usually positive to supravital staining with various acidic and basic dyes.

4. Vitamin A was determined in some granulocytes of Asteroidea, Polychaeta, Crustacea, etc. Vitamin C was nearly always found in the granulocytes. This substance occurred as round or rod-like granules similar to the mitochondria.

5. Glutathione was detected in the brown amoebocytes of Brachiopoda. It is supposed that this substance would form the oxido-reduction system in the cell with vitamin A and vitamin C.

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It is a pleasure to express my hearty thanks to Dr. S. HATAI for his kindness in reading and criticizing the manuscript in spite of his precious time. I am indebted to Dr. Y. WATANABE for his encouragement and advice during the course of the work, and to Mr. S. OKUDA for the kind identification of Polychaeta used in this experiment. I also wish to thank Dr. S. SUGIYAMA for his helpful advice on many matters.

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# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## XI. SUPPLEMENTARY OBSERVATIONS ON THE CYTOPLASMIC INCLUSIONS OF RED COLOURED CORPUSCLES IN THE BLOOD OF SOME MARINE INVERTEBRATES<sup>1)</sup>

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(With five figures)

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In the present paper, lipoids, vitamins, etc. as the content of the granular inclusions of red cells and the fine structure which is demonstrable by the method of TOMITA and others of silver impregnation are principally discussed. The materials used in the experiment consist of a part of those which have been the objects of my previous papers (OHUYE, 1937 a, 1938 a). The methods of investigation are given also in these papers.

The nature of granular inclusions of red blood cells found in some marine invertebrates has been reported in the previous papers (OHUYE, 1937 a, b). As is seen in one of them (1937 a), the staining of these bodies with Sudan III ended in failure except in the cases of *Urechis* and *Thalassema*. In the present investigation, however, it succeeded with the red cells of the following animals; *Physcosoma scolops*, *Terebella* sp. (*debilis*?), *Glycera chirori*, and *Travisia japonica*. That is, the refractive, round or oval granules which have a dancing and quivering movement, and vary in number and size, are stained deep red or orange red with Sudan III. Brown granules of *Arca inflata* and *Caudina chilensis* were negative to the staining of Sudan dyes in the present as in the former experiment. They are, however, positive to the supravital staining of indophenol blue and to the staining of Nile-blue sulphate—acetic acid, after the alcohol fixation, showing the presence of lipoidal substance. The Nadi-reaction is positive without exception in these bodies. The CARR and PRICE blue, due to the presence of vitamin A, was found only in the granular inclusions of some erythrocytes of *Travisia* upon rare

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occasions. On the contrary, vitamin C is found almost constantly as a part of granular inclusions; it is found in them as a few, small and round granules (*Caudina*); as a crescent or rod-like granule (*Travisia*: Fig. 1, b & c) or as a single, round granule (*Phycosoma*, *Terebella*, *Glycera* and *Arca*). Besides appearing in such granular inclusions, vitamin C granules appear in the cytoplasm of red cells occasionally. In the majority of cases these granules are difficult to distinguish from those of the preexistent granular inclusions because of their similar appearance, but a remarkable increase in number makes it clear that the newly appeared granules of vitamin C do exist (Fig. 2). This is quite analogous to the relation between neutral red bodies and induce granules. In the case of *Caudina*, the differences of the two granules are very distinct because the granular inclusion of

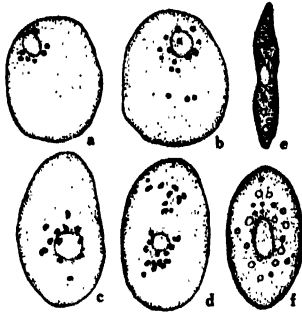


Fig. 1. Red blood cells of *Travisia*. ca. 1,200 $\times$ .

a-d. Cells with vitamin C granules.

e & f. Lateral (e) and front view of cell with neutral red bodies (black).

red cells consists of single, or, very rarely, double, large, and brown bodies. In the red cells of *Caudina* in prolonged captivity (about three weeks), the vitamin C granules are found only in the granular inclusion (Fig. 3, g & h) while they appear in the cytoplasm of newly captive of animals (Fig. 3, a-f). In the latter case, from five to thirty or more granules of vitamin C could be observed. They are fine, round or bacillus-like in shape, and usually scattered throughout the cell body, but occasionally they surrounded the nucleus (Fig. 3, b, c & e), or the brown body (Fig. 3, d). The shape and arrangement of these granules are quite similar to mitochondria which are demonstrable by the suprapital staining of Janus green B. BOURNE (1935) stated that most of the cells obtained from animals suffering from scurvy had contained constantly a very reduced

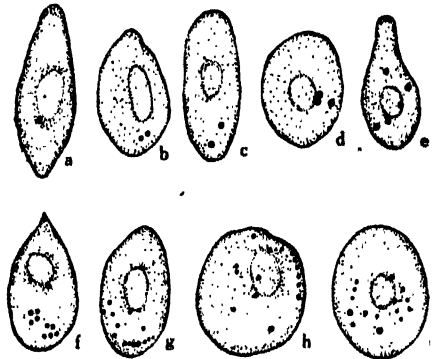


Fig. 2. Red cells of *Terebella* with vitamin C granules. ca. 1,000 $\times$ .

number of the vitamin C granules. The marked decrease in the number of vitamin C granules in the present case, therefore, may be due also to scurvy. As a matter of fact, the animals in prolonged captivity show scorbutic symptoms such as haemorrhages beneath the skin, mucous membrane, etc., a spongy condition of the body wall, anaemia and so forth.

The red cells of *Siphonosoma cumanense* are hyaline and homogeneous, and it is difficult to find the granular inclusion except as a single, round, refractory granule which is also positive to staining with Sudan dyes, the Nadi-mixture, and the reduction of silver nitrate. Vitamin C granules appear throughout the cytoplasm, and they are similar to those found in the red cells of healthy *Caudina*.

I have explained in a previous paper (OHUYE, 1937 a) that the granular inclusions are the constant component of the red cells of eight invertebrates, and that they are positive to the Nadi-reaction, and blackened with osmic acid with no exception. By the present experiment it also became clear that they show a constant fatty nature and are positive to the reaction of vitamin C. The CARR-PRICE reaction of vitamin A was also determined in these bodies of occasional cells, while attempts to detect glutathione always brought a negative result. I suppose, accordingly, that these granules would possess some relation to mitochondria, which are supposed to consist of lipoids, proteins, vitamins A and C, glutathione, ferments, etc., as was suggested by BOURNE (1935) and JOYET-LAVERGNE (1935); and that they would play a role in cellular respiration, as do mitochondria.

I reported to have made (OHUYE, 1937 a) a silver impregnation of the red blood cells of some invertebrates, using the method of TOMITA and others, and that a pattern similar to 'amphibian type' had been obtained in every case. In the present investigation, that method was applied to the red cells of *Travisia japonica*, *Glycera chirori*, *Sipunculus nudus*, and *Siphonosoma cumanense*. The red cells of *Travisia* showed either the amphibian type or droplet type (Fig. 4). The next two animals also possessed the pattern belonging to the amphibian type. The last animal,

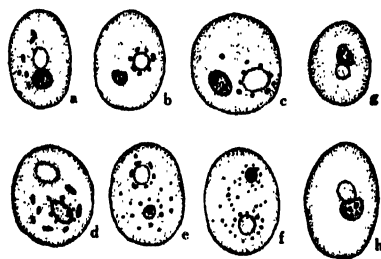


Fig. 3. Red cells of *Caudina*. ca. 1,000 $\times$ .

a-f. Red cells of newly captive of animals.

g & h. Red cells of animals in prolonged captivity.

however, showed a somewhat specific pattern. Needless to say, the granular inclusions are constantly positive to silver impregnation throughout



Fig. 4. Silver impregnation of red cells of *Travisia*. ca. 1,000 $\times$ .

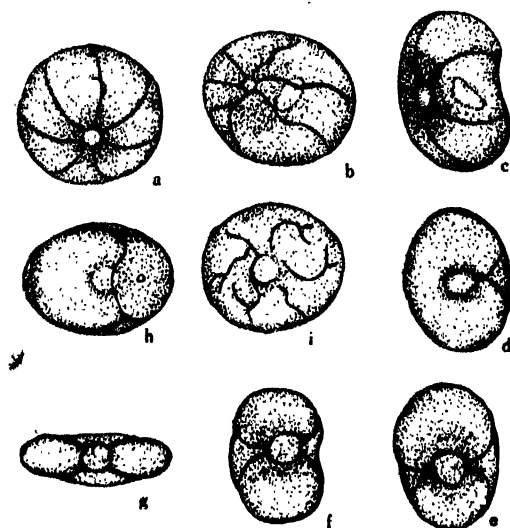


Fig. 5. Silver impregnation of red cells of *Siphonosoma*. ca. 1,500 $\times$ .

all the animals. The typical pattern of *Siphonosoma* is similar to the shape of a cart wheel; namely six silver impregnated rays which correspond to the spokes of the wheel radiate from a centre, at geometrically regular intervals, and with no branching (Fig. 5, a-c). The centre from which the spokes radiate is usually a small circle which is also impregnated with silver. A pattern whose spokes are reduced to 2-4 in number is also occasionally found (Fig. 5, d-h). Occasional cells possess an irregular pattern too (Fig. 5, i), owing, perhaps, to the unsatisfactory fixation. Partial haemolysis due to the action of saponin, hypotonic salt solution, etc. also causes the appearance of irregular patterns.

The patterns just mentioned above should not belong to any type of KAWABE's classification which I have mentioned in a previous paper (OHUYE, 1937 a). These are more or less similar to the 'transitional form of irregular type,' but such unbranched and geometrically regular patterns are not found in that form. It seems to me that the pattern of *Siphonosoma* should be recognized as specific to these animals, or, at least, that this is a new type of which no previous description is found.

The haemoglobin in the blood of *Travisia* is contained not only in the red cells, but also in the blood plasma. This is evident from the fact that the blood freed from the corpuscles by centrifugal separation shows

also transparent and deep red colour. There are, of course, many worms which have haemoglobin dissolved in the plasm, but with no red cells, while others possess blood of exactly the reverse nature. The blood of *Travisia*, therefore, shows a transitional stage from the former to the latter. I sought to find some specific blood cells which may occur in such special blood, but my efforts resulted in failure. The stages in the maturation of red cells were quite similar to those of the vertebrates.

The red cells of *Travisia* are usually oval and slightly biconcave disks (Fig. 1), measuring about  $20\ \mu$  in longer-,  $15\ \mu$  in shorter diameter, and  $4\ \mu$  in thickness. They possess, naturally, a single, round or oval, and centrically localized nucleus. However occasional red cells are so small that their diameter is approximately half of that of usual cells, and no nucleus is found in the cell body. Whether these cells are the so-called erythroplastid found by ROMIEU (1923) in the blood of *Thalassemia* and *Magelona*, or the mere fragments of red cells, is yet uncertain. Intracellular crystallization of haemoglobin was also found in the red cells of *Travisia*. The shape and state of aggregation of crystals are quite like those of *Arca* of which I have previously given information (OHUYE, 1937 a). The neutral red bodies were from two to five in number (Fig. 1, e & f). The preexistent granules were easily supravitaly stained with brilliant cresyl blue, Nile-blue sulphate, etc.

#### SUMMARY

The granular inclusions found in the red cells of *Travisia*, *Glycera*, *Terebella*, *Physcosoma*, *Sipunculus*, *Siphonosoma*, *Caudina*, and *Arca* were constantly positive to the reactions of fats, oxidase, and vitamin C. Some of them showed also the reaction of vitamin A. Pattern which seems to be specific to the red cells of *Siphonosoma* was demonstrated by the method of silver impregnation. Haemoglobin is found in both the plasm and the red cells of *Travisia*.

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# THE RÔLE OF THE VISUAL SENSE ORGAN IN AGGREGATION OF *PLOTOSUS ANGUILLARIS* (LACÉPÈDE), WITH SPECIAL REFERENCE TO THE REACTIONS TO MIRROR

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(With two figures)

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*Plotosus anguillaris* (LACÉPÈDE) is frequently found in the summer month in shallow water near the beach. Having a school habit, a single dip with a net may take great numbers. Although the body length of each individual of a school is usually nearly equal, but as the sex-ratio, nothing has been observed. When brought into the laboratory, these fishes assemble to form a black mass in the aquarium, usually crowding to the side. However, when shelters, as stones are supplied, they take opportunity and quietly rest there. During the winter, the water temperature is about 11°C, the fishes seem to lose aggregating behaviour.

The problem concerning the aggregating behaviour of the fish is studied from various angles, and as the first attempt, a study is made on the sense organs as bearing upon this behavior. In this work, the rôles played by the vision of fish in the formation of aggregation is the chief aim.

The present experiments were based mainly on fishes of 7-8 cm. in body-length. They were collected from Simoda Bay and kept in two tanks of the same size, 1 × 1 × 1.5 m. During the course of the experiments, these fishes were fed by worms or meats.

As this place I wish to express my gratitude to Dr. S. HATAI for his very valuable and kind criticisms given me during the course of this investigation.

## 1. THE RÔLE OF THE VISUAL SENSE ORGAN

PARR ('27), in a study of schools of pelagic fishes, concluded that the behaviour of individuals is controlled by visual stimulation. According to BOWEN ('31), the eye is an important sense organ in the formation of the catfish aggregation seeing that aggregation is broken up in about a minute after the light is removed. SPOONER ('31) studied the schooling

behaviour in the bass and found that sight is a prime factor. It can be concluded from these observations that the eyes are extremely important for the aggregation of the fish.

My first experiments were performed to test the relation of the aggregation to the presence of light. All of the experiments were done in a dark room. A 60 Watt Mazda electric lamp which could be switched on and off at will was placed over the center of an aquarium,  $69 \times 41 \times 36$  cm., in size and containing several fishes. These fishes were left for definite intervals in the dark, and were observed as the light was flashed on. For convenience, the degree of aggregation is recorded in terms of the following five stages as employed by BOWEN ('31):

Stage I. Close aggregation — fishes in one or two compact groups.

Stage II. Loose aggregation.

Stage III. Slight grouping — nearly one half of individuals formed a compact group.

Stage IV. Mostly scattered — less than one half of individuals formed a compact group.

Stage V. Completely scattered.

The results obtained are presented in Table 1.

TABLE 1.  
*Stages of aggregation of 50 normal fishes after given  
intervals of dark.*

(Water temp.  $14^{\circ}\text{C}.$ , April 6th)

Interval	Stage	Time (seconds) required to reform a compact mass
10 s.	II	9
20 s.	II	8
30 s.	II	14
40 s.	II	12
50 s.	III	10
1 m.	III	9
1 m. 30 s.	III	15
2 m.	V	20
2 m. 30 s.	V	30
3 m.	V	22
4 m.	V	20
5 m.	V	31

Similar results were obtained for the fishes of other sets. These results show that there was no case of Stage I, and Stage II was observed only at a short period in the dark. When these fishes were left in the darkness for two minutes and more, they scattered in the aquarium. Close

aggregation, however, took place shortly after the light was turned on. Accordingly, aggregation is followed by the persence of light.

That the blinded catfish do not aggregate has not only been described by EDDY ('25) and BOWEN ('31), but has been repeatedly confirmed by my own observations. Thirty fishes blinded by means of a hot needle, usually did not aggregate even in the light, though they would sometimes turn to follow another fish when closely passing by. Seven days after this operation, these fishes were tested by the same method described above. The results are shown in Table 2.

TABLE 2.

*Stages of aggregation of 30 blinded fishes after given intervals of dark time.*

(Water temp. 19°C)

Interval	Stage	Interval	Stage
10 s.	IV	1 m.	IV
20 s.	V	2 m.	V
30 s.	V	3 m.	IV
40 s.	II	4 m.	V
50 s.	V	5 m.	V

From the above table, it is noted that the degree of scattering is not varied directly with the given intervals of dark, in marked contrast to the case of the normal fishes (see Table 1).

That the catfish scattered in the dark leads to suspect that it does not aggregate in the dark night. BOWEN ('31) declared that the catfishes show no aggregation during the darker part of the night, but a gradual increase in the degree of aggregation occurs with increasing dawn.

My observations were made at hourly intervals throughout the night (Oct. 11th '36) on the behaviour of 50 normal fishes in the laboratory. There was no moon. They scattered in the aquarium during the whole time from 8 p.m. to 5 a.m. the next morning, but gradually formed a loose aggregation as it began to grow lighter and finally assembled at about 5.20 a.m., and remained thus, the entire day. The sea catfishes, therefore, may scatter in the dark night in their natural habitat as can be judged from laboratory observations.

All of the above results indicate that vision is very important in the formation of aggregation, which otherwise generally breaks up.



## 2. THE REACTIONS TO THE MIRRORS

From the foregoing results, it appears that this sea catfish might show reaction to the mirror image. SPOONER ('31) from his studies on the schooling of the bass finds that this fish reacts to the mirror, and that the reaction is most remarkable in one isolated individual — progressing less as the number increases. But, however, he also noticed that no attention was paid to such a small mirror as one that reflects only the eyes or the head of the fish. Moreover, the distribution of fishes in the tank was affected by the presence of the mirror. In connection with these results, it seems to me very interesting to observe the reactions of this sea catfish to the mirror.

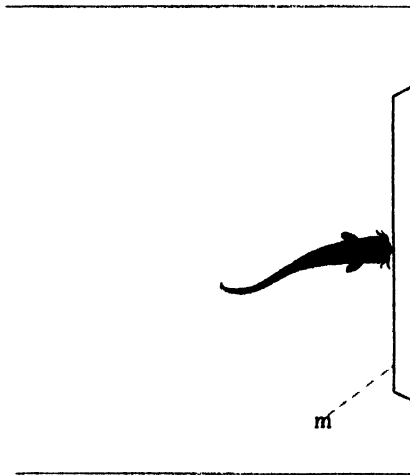


Fig. 1. Schema showing the reactions of the fish to the mirror, m.

At first I watched the reaction of a single fish to a rectangular mirror, 18 × 13 cm. in size, which was placed against one side of a wooden aquarium measuring 41 × 27 × 20 cm. When the fish was transferred into this aquarium with the mirror, it swam readily to the mirror spending much time swimming to and fro in rapid zigzag motion across the mirror surface with its face close against the glass, as though attempting to pass through it (Fig. 1). The behaviour exhibited by these fishes was always essentially

TABLE 3.

*The duration of the reactions of a single fish to the mirror.*

(Time of each observation is 5 minutes. Water temp. 19°C)

No. of specimen	Shortest and longest duration	No. of times of reactions	Sum
I	2 s.—2 m. 30 s.	4	2 m. 50 s.
II	40 s.—1 m. 40 s.	2	2 m. 20 s.
III	5 s.—2 m. 32 s.	3	3 m. 51 s.
IV	1 m. 15 s.—2 m. 57 s.	2	4 m. 12 s.
V	1 m. 20 s.	1	1 m. 20 s.
VI	10 s.—1 m. 5 s.	5	3 m.
VII	5 s.—55 s.	8	2 m. 15 s.
VIII	1 m.—2 m. 30 s.	2	3 m. 30 s.
IX	35 s.—1 m. 15 s.	4	3 m. 18 s.
X	5 s.—1 m. 26 s.	4	2 m. 5 s.

of the kind described above, regardless of body length and sex. But, the period during which the fish reacts to the mirror varied with each individual. I examined the duration of the reaction to the mirror and obtained the results shown in Table 3.

These results show that the fish, as might be expected, was greatly attracted by the mirror in a similar manner as the case of the bass.

Next I tested with the same methods and under the same conditions whether the mirror exerts less attraction to the fish as the individual number increases. The results were Tables 4-6.

TABLE 4.

*The duration of the reactions of two fishes together to the mirror.*

No. of set	Shortest and longest duration	No. of times of reactions	Sum
I	33 s.—2 m.	2	2 m. 33 s.
II	4 s.—1 m. 30 s.	6	2 m. 21 s.
III	30 s.—1 m. 10 s.	2	1 m. 40 s.
IV	40 s.—1 m. 27 s.	2	2 m. 7 s.
V	7 s.—2 m. 8 s.	3	3 m. 19 s.
VI	21 s.—56 s.	3	2 m. 10 s.
VII	10 s.—1 m. 12 s.	4	2 m. 5 s.

TABLE 5.

*The duration of the reactions of four fishes together to the mirror.*

No. of set	Shortest and longest duration	No. of times of reactions	Sum
I	5 s.—1 m. 11 s.	5	3 m. 1 s.
II	6 s.—47 s.	5	2 m. 4 s.
III	2 s.—31 s.	12	2 m. 1 s.
IV	15 s.—40 s.	4	1 m. 37 s.
V	18 s.—1 m. 10 s.	4	2 m. 41 s.
VI	10 s.—1 m. 5 s.	5	2 m. 24 s.
VII	2 s.—45 s.	12	1 m. 15 s.

TABLE 6.

*The duration of the reactions of ten fishes together to the mirror.*

No. of set	Shortest and longest duration	No. of times of reactions	Sum
I	15 s.—25 s.	2	40 s.
II	7 s.—30 s.	4	1 m.
III	12 s.—20 s.	3	46 s.
IV	3 s.—16 s.	4	37 s.
V	5 s.—18 s.	3	34 s.

From these results, it will be seen that the increase of individuals decreases the intensity of the fish's reaction to the mirror. With the increase in number of fishes, they swam most of the time in a mass and thus they paid less attention to the presence of the mirror than a single one did. Hence the reactions to the mirror are best seen in a single specimen isolated from the rest. But, it must be noted that a single fish did not show remarkable reactions to the mirror when it was restricted in the aquarium with the mirror for a period longer than thirty minutes.

Employing eight fishes a series of experiments was then run in order to compare the duration of the reactions of a single fish to three mirrors of different sizes. Each fish was observed for five minutes with the method described above. The results are as follows.

TABLE 7.  
*Duration of reactions to three mirrors of different sizes.*

No. of specimen	Size of mirror 8×6 cm. (reflects the whole body)	4×3 cm. (reflects half of the body)	3.5×2 cm. (reflects the head alone)
I	1 m. 3 s.	1 m. 20 s.	17 s.
II	1 m. 37 s.	1 m. 23 s.	28 s.
III	33 s.	12 s.	23 s.
IV	1 m. 22 s.	8 s.	3 s.
V	37 s.	20 s.	40 s.
VI	51 s.	6 s.	4 s.
VII	1 m. 38 s.	1 m. 37 s.	1.5 s.
VIII	1 m. 58 s.	31 s.	4 s.

The results recorded here corroborate the investigations performed by SPOONER ('31) on the bass: the complete image is not necessary to bring out this sort of reaction. But the duration of reactions to the mirror was reduced with decrease in the size of the mirror.

In connection with the experiments on the mirror, I tested the behaviour of this fish to plain glass or porcelain plates with smooth surface.

To a rectangular glass plate, 18×13 cm. in size, placed against one side wall of the wooden aquarium, the fishes gave no reactions, excepting one case in a total of twenty observations. To dark glass plate or porcelain plates, they paid little attention.

The evidence of the facts indicates that this characteristic behaviour described above is not evoked by any sort of plates without clear images, as no reactions occur. The positive evidence for this is that this behaviour is not observed on the blinded fishes and the intensity of this reaction is

not affected by the removing of all barbels<sup>1)</sup> about the mouth of the fish. Accordingly, it may be concluded that the mirror image exerts an influence identical with that of the presence of another individual, evoking the aggregating reaction. But, it must be kept in mind that this fish shows far less decided reactions to the mirror laying on the floor of the aquarium, thus differing from the case of the bass (SPOONER, '31). This catfish usually swam over the mirror on the floor without paying attention. This subject needs further investigations before any definite conclusion can be drawn, but it seems to be highly probable that the slight attention of the fish to the mirror on the floor is due to the movement<sup>2)</sup> of the eye of the fish.

BOWEN ('31) pointed out that the catfish follows a moving object in a way which, if continued, would result in the formation of aggregation. SPOONER ('31), however, has concluded that the movement of the object is not essential for evoking the schooling behaviour of the bass, since a live fish reacts to a dead one. In order to ascertain this subject on the present fish, I performed the following observations.

When a small object such as a leaden ball, stone or a dead fish was drawn through the water containing the fishes, they swam<sup>3)</sup> in pursuit of it in a similar manner as that described by BOWEN ('31). But, these fish did not pay any remarkable attentions to such objects when they were not drawn through the water. Though this fish approached to a dead one, as noted by SPOONER ('31), the fish soon escaped from the dead one and did not remain to lie alongside it. Furthermore, this fish did not show any reactions to a painted picture which closely resembles a fish and is stood up against one wall of the aquarium, covered by a thin glass plate.

Next, I carried out my investigations by the use of a small glass box, 17×6×10 cm. in size, furnished with a covering plate in order to avoid the tactile impulse induced by the direct contact to the body of the fish. This small glass box in which sea water and a live or dead fish were contained was placed in the wooden aquarium described above. After this procedure, I brought the fish into this wooden aquarium and observed the reactions of the introduced fish to this small box. The introduced

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<sup>1)</sup>I reported already on the barbels of this fish (Sci. Rep. Tôhoku Imp. Univ., Biol. Vol. 11, No. 3, '37).

<sup>2)</sup>SCHICKE, O. S. examined the movement of the eyes of *Amiurus nebulosus* LES. (Zool. Jahrb., Abt. allg. Zool. u. Physiol., Bd. 38, 49-112. '21).

<sup>3)</sup>This reaction was most distinct in the small fish of 3-4 cm. in body-length.

fish gave reactions similar as that induced by the mirror to this small glass box in which a live or dead fish was confined (Fig. 2). It is

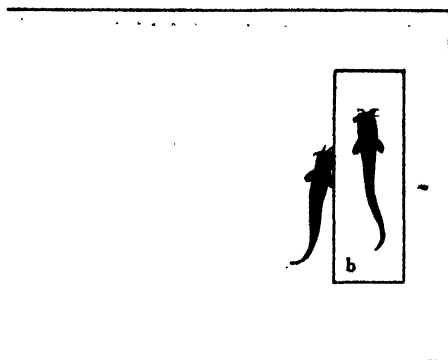


Fig. 2. Schema showing the reactions of the fish to a glass box, b, in which a live or dead fish is confined.

interesting to note that a live fish in this glass box did not always follow in pursuit of the outer fish. I measured the period during which the fish reacts to this glass box. Each observation was done for five minutes. The average duration obtained by ten observations on the reactions to the box containing a live fish was 54 seconds, and the same to a dead fish was 17 seconds. It is, therefore, probable that this catfish is excited more

powerfully by the movement of the live fish than either the dead or imitated fish.

As for the last test, an attempt was made to compare the frequency with which the fish reacted to the mirror with that to the glass box containing a live fish. By this means an indication may be given as to whether the presence of the mirror made any difference. A rectangular mirror, 18×13 cm. in size, was placed against one wall of the wooden aquarium and a glass box used in the former investigation was placed on the floor about 35 cm. apart from this mirror. A fish was then introduced into this wooden aquarium, and the duration of the reactions of this fish to the both mirror and glass box containing a live fish was observed for five minutes. This investigation was repeated with different specimens with the similar results as are shown in the following table.

There is a greater probability that the fish will swim in front of the mirror than in front of the glass box containing a live fish. It is impossible to interpret definitely these results, but this attraction of the mirror seems to be probably due to fact that the mirror image follows perfectly with the movement of the fish in the aquarium, while the fish restricted in the small glass box does not always react in pursuit of the outer fish.

Taking advantage of that the fighting fish is excited by the mirror image, BRECHER ('33) and LISSMANN ('33) determined the "moment" or "Zeiteinheit" of this fish. But, I could not obtain the "moment" on

TABLE 8.

*Duration of the reactions of ten fishes to the both mirror and glass box containing a live fish.*

No. of specimen	Mirror	Box
I	1 m. 43 s.	33 s.
II	1 m. 29 s.	37 s.
III	1 m. 52 s.	32 s.
IV	1 m. 43 s.	1 m. 23 s.
V	2 m. 31 s.	25 s.
VI	1 m. 42 s.	6 s.
VII	1 m. 53 s.	31 s.
VIII	1 m. 23 s.	16 s.
IX	33 s.	4.5 s.
X	3 m. 10 s.	2 s.
Average	1 m. 48 s.	27 s.

this sea catfish by the same methods as that worked by the previous two authors, owing to the fact that this did not react decidedly to the mirror standing closely near to the outer wall of the glass aquarium. This subject will be discussed in the near future.

#### SUMMARY AND CONCLUSIONS

1. The vision plays a leading rôle in the aggregation of the sea catfish since the normal fishes fail to aggregate in the dark and the blinded ones do not come together into a compact mass.

2. This fish reacts to the mirror stood up against one wall of the aquarium: the fish spends much time in swimming by the surface of the mirror in rapid zigzag motion, as though to pass through it.

3. This characteristic behaviour is always essentially of the kind described above, regardless to the body length and sex of the fish.

4. The time during which the fish reacts to the mirror is shortened with the presence of the other fish and with the decrease of the size of the mirror. For evoking this reaction, however, a complete image is not necessary.

5. It is interesting to note that this fish pays slight attention to the mirror laying on the floor of the aquarium. And this fish does not show any reactions to any sorts of plate without clear image.

6. This fish is more attracted by the movement of a live fish than either a dead or imitated fish.

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A BRIEF OBSERVATION ON THE BIOLOGY OF HERRING  
(*CLUPEA PALLASII* C. & V.) WHICH MIGRATES INTO  
LAKE OBUCHI, A BRACKISH LAKE IN  
AOMORI PREFECTURE, JAPAN<sup>1)</sup>

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(With eight figures)

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1) INTRODUCTION

Among the races of Japanese herring which were studied by FUJITA and KOKUBO (1923, 1926, 1927) a race called Hinuma herring particularly interested us, because of the fact that the Hinuma herring forms a distinct race, contrasting with the Hokkaido herring which constitutes the bulk of Japanese herring. Furthermore, the fact that it regularly migrates into Hinuma, a brackish water lake, for the purpose of spawning, doubled our interest.

Hearing that the Obuchi herring also migrates into the brackish Lake Obuchi, we naturally wondered whether it might be the same race as that of Hinuma or whether it may likewise spawn in Lake Obuchi. To resolve this question we made a preliminary observation of the Obuchi herring and its environmental conditions. For the purpose of comparison, the Asamushi herring from Aomori Bay were also studied.

In the present paper some numerical data have been given with reference to the racial characteristics of these herrings, but the final decision may be made only after adding further data.

We here tender our hearty thanks to Prof. S. HATAI for his kindness in looking over the present paper.

2) MATERIAL AND METHOD

*Material.* The Obuchi herring (Fig. 1) were obtained from Obuchi Village where they are abundantly caught in Obuchi River. Two lots consisting of 82 fishes in all were obtained, the first one was collected

<sup>1)</sup>Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 150.



by TEZUKA on Dec. 27th 1936, and the second was collected and sent to us by Mr. Y. MAITA of Obuchi to whom our thanks are due. The first lot of 10 fishes was caught on the morning of Dec. 27, while the second of 72 fishes was collected during the period from Jan. 25 to 27th, 1937.



Fig. 1. Herring (*Clupea pallasii* C. & V.) of Lake Obuchi.

The Asamushi herring was collected in mid-April 1935 in the vicinity of our laboratory. This herring is not abundant, but frequents regularly from year to year, and is caught by fishermen with a gill net which has the mesh of about 49 mm.

**Method.** FUJITA and KOKUBO (1927) employed a method derived from both HEINCKE (1878) and BROCH (1908), the former being referred to the Scandinavian investigator NILSSON. In Studying the English herring, ORTON (1916) followed the scheme of the Board of Agriculture and Fisheries. In studying the herring of the North Sea and Ratezat, JOHANSEN (1923, 1924) measured the number of vertebrae, keel scale, and ray of fins, but disregarded the position and length of fins, according to the current view that, characters which remain constant throughout the whole life of fish is to be preferred to define races. Most of the above methods essentially consist in the representation of measurement by an index which represents the ratio between the total length of the body and each measurement. In the present study FUJITA and KOKUBO's (1927) method with some modifications was used. The scheme of the characters studied by us is as follows:

- 1) Total length of the body (T)
- 2) Body length (T—cd)

- 3) Caudal length (cd)
- 4) Sex
- 5) Length of dorsal fin (D)
- 6) Dorsal fin ratio (D/T)
- 7) Lateral length of the head (lcpl)
- 8) Head ratio (lcpl/T)
- 9) Weight of fish (Wt)
- 10) Weight of ovary (Wt.O)
- 11) Ovary ratio (Wt.O/Wt)
- 12) Age due to scale.
- 13) Number of keel scale ( $K_2$ )

For the procedure for measuring these characters, the readers are referred to FUJITA and KOKUBO (1927). Of these 13 items the Wt.O/Wt is not so far studied. This was first tried this time with the object of expressing the degree of maturity. As is evident from their results the most useful measurement was that of the length of head and the number of keel scale. Even only from these two factors the local races can be defined to a certain extent.

To define one race from other we adapted the statistical method used by BROCH (1908). He compared the variation curves by calculating the average of an index and its probable error ( $0.6745 \sigma/\sqrt{n}$ ), basing on the general principle that when two variation curves are mathematically different, the relation between the average and probable error should be;

$$G_1 - G_2 \geq 3\sqrt{SF_1^2 + SF_2^2}$$

Where  $G_1$  and  $G_2$  signify two averages.  $SF_1$  and  $SF_2$  being the probable error of  $G_1$  and  $G_2$  respectively. From this method whether the three folds of probable error are smaller or larger than the difference between two averages will be counted.

FORD (1928) also made comparison of two averages by a similar method. In his case, however, the magnitude of 'standard error' ( $\sigma/\sqrt{n}$ ), instead of probable error, was compared to the difference of two mean values. The formula employed by him was,

$$(m_2 - m_1) > 2\sqrt{\sigma m_2^2 + \sigma m_1^2}$$

Where  $m_2$  and  $m_1$  designates two mean values, and  $\sigma m_2$  and  $\sigma m_1$  are respectively the standard errors of  $m_2$  and  $m_1$ . Actually the both methods well coincide as the probable error is 1/0.6745 times the standard error the three times probable error is approximately the same as twice the standard error.

## 3) RESULTS

## a) The Herring and its Environment.

*Herring invading into lake.* As far as the present authors are aware there are several populations of herring which invade the brackish lakes of Japan. Of these herrings two local races were reported by FUJITA and KOKUBO (1927) from Lakes Tonnai (Karafuto) and Hinuma.

One of the herring studied here has been found in Lake Obuchi as previously stated. Locally this herring has long been known because of its regular frequentation. The catch of this herring appears to have been fairly great until some twenty years ago. Since then it has become of but minor economic importance, as the catch can supply no more than Obuchi Village. Though the causes of this depletion are not certain, overfishing might have been one of the contributing causes. TAMURA (1932) reported the herring from Lake Takahoko, a little north of Lake Obuchi. But this herring seems to be very scanty and irregular in yield.

*Herring frequenting Asamushi.* Owing to the fact that no herring is ever caught in Aomori Bay except during the spawning season (April) it seems certain that this herring migrates from the Tsugaru Strait. Concerning the movement of herring in Aomori Bay it has long been believed that the school from Tsugaru Strait migrates along the west coast of the bay towards the south. On reaching the head of the bay they turn eastwards and then northwards, and finally go out to the strait passing Noheji Bay.

*Hydrography of Lake Obuchi.* Along the Pacific coast of Shimokita Peninsula, Aomori Prefecture, are found five shallow lakes of varying sizes. Excepting the smaller two, each of the other three lakes are in communication with the Pacific Ocean through a narrow outlet which is temporarily closed on account of the heavy east wind prevailing in one or the other season of the year. The closure of the mouth does not last long as it is drained away by the flooding of the lake or by some artificial means. The water of all these three lakes is brackish, the salinity varying according to the amount of inflowing water.

Among these lakes the northernmost is Lake Obuchi, of about 3.74 sq. kilometers in area, and the circumference measuring about 12 kilometers. Its shape is like a rectangular triangle, the longest side stretching east and west, and opening into the sea at the east corner. The mean depth of the lake is probably 2 meters or thereabout. In the lake are two areas each of which is surrounded with an isometric depth of 5 meters,

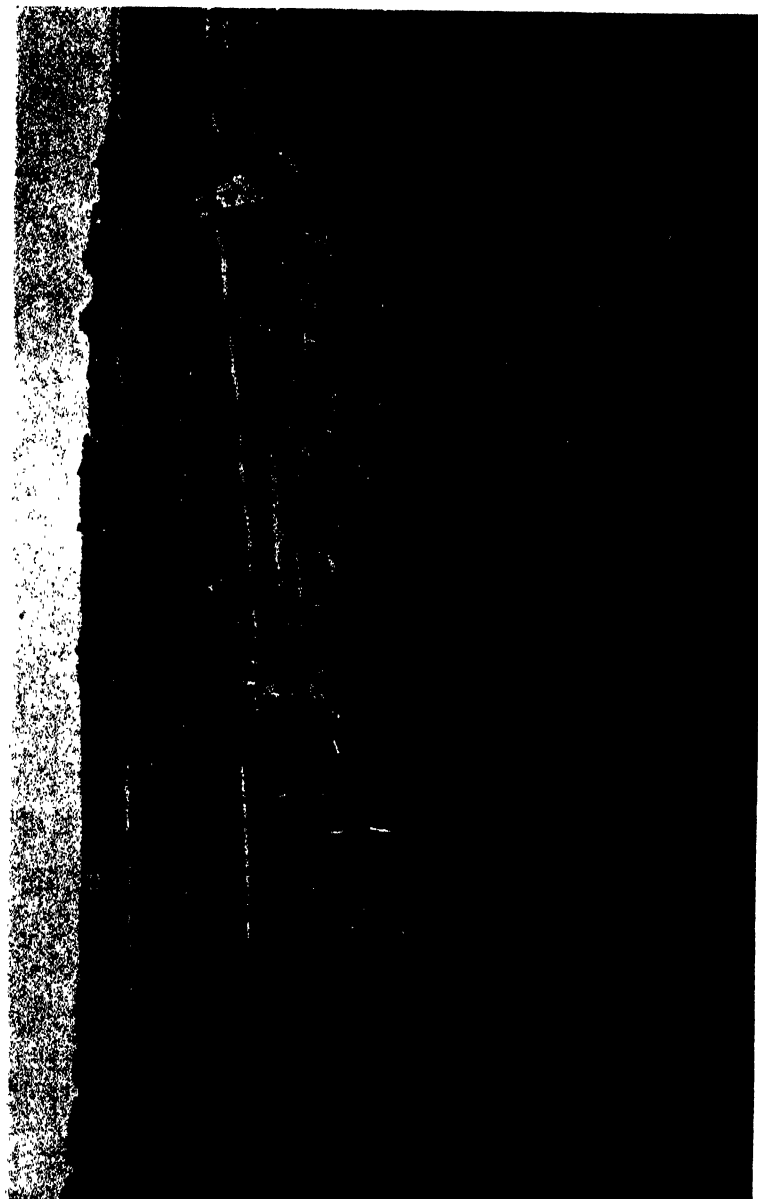


Fig. 2. Herring-net.

one lies a little to the west of the centre of the lake and gives the maximum depth of 5.5 meters in its centre. The east corner of the lake extends into the Obuchi River which has a maximum breadth of about 300 meters and runs about 1.5 kilometers towards the sea. The bottom of the lake largely consists of mud which gradually merges into sand towards the river.

The level of the lake is under constant influence of the tide, its diurnal change usually ranging between 15 and 30 cm. Existence of a tidal stream through river and lake is recognized from the fact that the waste material in the river reaches the centre of the lake when the tide floods.

Observation I. The first observation of the water temperature was made by us during the 27th-29th of December, 1936, at a station near the trap-net of herring (Fig. 2), with the following result.

Date (Dec. 1936)	Weather	Hour	
		10.00 a.m.	4.0 p.m.
27th	fine	3.5°C	3.2°C
28th	blizzard	3.0°C	3.0°C
29th	blizzard	2.9°C	2.8°C

The above observation shows that these are the temperatures of the river just when the herring began to migrate into the lake. From this time onwards the herring continues incoming migration through the coldest season of late February when the lake freezes. On March 13, 1937 an occasional observation was made at the above station and the water temperature was found to have sunk to  $-0.5^{\circ}\text{C}$ . Thus it was found that the migration occurs during the coldest season of the year. During this season the temperature of the river showed  $3.5^{\circ}\text{C}$ . at the beginning and sank as low as  $-0.5^{\circ}\text{C}$ . at the end.

Observation II. The second observation was made on April 4-5th. The results are tabulated as the following table.

At the time of this observation the incoming migration of herring just ended, the school culminating in the uttermost of the spawning. Therefore the above results can be considered as the conditions of the spawning season. Apr. 4th of this year was the eighth day after the complete thaw of the ice, and the opening of the river was fully drained on account of the thawing flood.

The temperature of the water which was about  $3.0^{\circ}\text{C}$ . in the preceding observation now ranged around  $5^{\circ}$ - $6^{\circ}\text{C}$ . Such low temperatures as  $2.5^{\circ}$

St.	Date	Hour	Weather	Air Temp. (°C)	Tide	Depth (m)	Bottom	Observed layer	Water Temp. (°C)	pH	Spec. grav. ( $\sigma_t$ )	Oxygen (cc per l.)	Remarks
St. A	Apr. 3	5.00 p.m.	Fine	5.3	Ebb.	0.5	Mud	—	7.5	8.2	6.88	7.32	Tide ebbing, 1 m. per min.
St. A	Apr. 4	6.00 a.m.	Fine	1.2	Ebb.	0.5	Mud	—	2.5	8.0	6.62	7.31	Tide ebbing, 0.1 m. per min. Shore frozen.
St. A	Apr. 4	10.00 a.m.	Fine	4.5	Interm.	0.58	Mud	—	3.8	8.2	6.69	7.74	Tide rising, 0.1 m. per min. Ice melted.
St. A	Apr. 4	1.00 p.m.	Fine	6.0	Flood	0.78	Mud	Surface	6.0	8.2	8.13	7.11	Almost stagnated.
St. A	Apr. 4	1.00 p.m.	Fine	6.0	Flood	0.78	Mud	Bottom	5.8	8.2	8.14	6.83	
St. A	Apr. 4	4.00 p.m.	Fine	6.2	Interm.	0.54	Mud	—	6.5	8.0	7.25	6.45	Tide ebbing, 0.2 m. per min.
St. B	Apr. 4	8.00 a.m.	Fine	4.2	Flood	0.6	Sand	0.3 m.	5.5	8.0	6.82	7.22	Some grasses at the bottom.
St. C	Apr. 4	9.30 a.m.	Fine	4.2	Flood	0.2	Rock	—	5.5	7.9	6.70	6.96	With Iwagai at the bottom.
St. D	Apr. 4	10.30 a.m.	Fine	5.0	Interm.	1.5	S. & M.	Surface	5.8	8.0	6.94	7.35	Spawning ground. (Grass flourishing at the bottom)
St. D	Apr. 4	10.30 a.m.	Fine	5.0	Interm.	1.5	S. & M.	Bottom	4.6	8.1	7.56	7.40	
St. G	Apr. 5	8.45 a.m.	Rain	6.8	Ebb.	4.0	Mud	Surface	5.8	8.0	7.99	7.67	Forel's scale, No. 7-8. Transparency, 2.5 m.
St. G	Apr. 5	8.45 a.m.	Rain	6.8	Ebb.	4.0	Mud	2.0 m.	5.8	8.0	7.91	7.27	
St. G	Apr. 5	8.45 a.m.	Rain	6.8	Ebb.	4.0	Mud	Bottom	5.8	7.9	8.01	7.22	
St. F	Apr. 5	9.30 a.m.	Rain	6.8	Flood	3.2	Mud	Surface	6.6	8.1	7.09	7.48	Forel's scale, No. 7-8. Transparency, 2.5 m.
St. F	Apr. 5	9.30 a.m.	Rain	6.8	Flood	3.2	Mud	1.5 m.	6.5	8.1	7.41	6.68	
St. F	Apr. 5	9.30 a.m.	Rain	6.8	Flood	3.2	Mud	Bottom	6.2	8.0	8.38	6.95	
St. E	Apr. 4	11.30 a.m.	Fine	6.2	Interm.	2.0	Sand	Mid-layer	6.0	6.6	—	7.94	Water greatly increased due to thaw. Speed 0.3 m. per min.

and 3.8°C. were measured in the early morning when the shore occasionally froze. At a station (St. D) where the spawned eggs were found, the temperature was 5.8°C. at the surface and 4.6°C. at the bottom. As can be seen from the above table there seems to be no regular horizontal distribution in water temperature.

The specific gravity of the water ranged between 1.007–1.008 at all stations. A slight tendency towards increasing salinity at the bottom was observed at three stations (St. D, St. G, St. F). The specific gravity of this season is certainly less than that of September which is given elsewhere. Such a decrease of salinity might surely be caused by the inflow of thawing water. The pH of water is roughly subject to the change of salinity, and ranged between 7.7 to 8.0, excepting the estuary (St. E) where it was 6.6. The oxygen content of the water was relatively high owing to the low temperature of water.

The above results suggest to us that the spawning of Obuchi herring takes place when the water temperature attains about 6°C., and when the salinity decreases down to about 1.007 or so, probably the minimum of the year.

Apart from our records above stated a particular observation was made by Mr. S. CHIBA of the Fishery Station on Sept. 9th, 1931, to his courtesy the following results are due. His observations were made along a straight line directed S-E from Futamata River to the S-W side of the lake, crossing the deepest point. Thirty four stations listed in the following table are recorded from the south east side to the Futamata River in order.

The points of interest which are raised by the following table will be stated as follows:

The temperature of the water of this lake varies around 18°–19°C in late summer. A slight tendency of indirect stratification observed at several points might be caused by the greater density of water which stagnates in the lower layer. Probably the surface water with a low temperature might be unable to sink below the bottom water because of its lower salinity, thus resulting in the indirect stratification.

The horizontal distribution of surface salinity of the lake is very uniform through all regions, showing but slight variations around 1.005 (ca. 7.6‰, 15°C.). Such a homogeneous distribution is merely the result of the absence of large inflowing river which dilutes the salinity. But as to the vertical distribution a distinct stratification due to salinity was observed, as for instance between St. 12 and St. 28. In these stations the salinity

St.	Surface		Intermediate layer		Bottom layer		Depth in m.
	Temp. C.	Sp. Gr.	Temp. C.	Sp. Gr.	Temp. C.	Sp. Gr.	
1	18.2	1.0045	—	—	—	—	0.22
2	18.2	—	—	—	—	—	0.53
3	18.5	1.0050	—	—	—	—	0.61
4	18.7	1.0057	—	—	—	—	0.74
5	19.0	1.0060	—	—	—	—	0.88
6	19.2	1.0060	—	—	—	—	0.87
7	19.3	1.0060	—	—	—	—	1.00
8	19.4	1.0057	—	—	—	—	1.19
9	19.4	1.0057	—	—	—	—	1.85
10	19.4	1.0057	—	—	—	—	4.72
11	19.5	1.0056	—	—	—	—	5.00
12	19.4	1.0056	19.9	1.0100	19.7	1.0175	5.20
13	19.3	1.0065	19.6	1.0050	19.7	1.0170	5.19
14	19.0	1.0055	19.5	1.0057	19.7	1.0170	5.00
15	19.5	1.0060	19.5	1.0055	19.7	1.0155	5.00
16	19.5	1.0055	19.5	1.0060	19.7	1.0160	4.90
17	19.5	1.0060	19.5	1.0055	19.9	1.0145	5.00
18	19.5	1.0055	19.7	1.0055	20.0	1.0125	4.70
19	19.6	1.0060	19.5	1.0060	19.8	1.0125	4.50
20	19.5	1.0055	19.7	1.0060	20.0	1.0125	4.50
21	19.0	1.0055	19.6	1.0060	19.8	1.0120	4.50
22	20.0	1.0055	—	—	20.0	1.0130	4.50
23	19.6	1.0054	19.8	1.0120	19.8	1.0130	4.30
24	19.8	1.0055	19.6	1.0058	19.8	1.0130	4.10
25	19.6	1.0055	19.6	1.0058	19.9	1.0132	4.20
26	18.6	1.0080	19.6	1.0051	19.7	1.0130	4.10
27	18.5	1.0024	19.6	1.0054	19.8	1.0130	4.00
28	18.8	1.0035	19.6	1.0050	19.8	1.0130	3.90
29	19.1	1.0052	19.5	1.0052	19.5	1.0063	3.70
30	19.2	1.0047	19.4	1.0055	19.4	1.0055	3.30
31	19.4	1.0050	19.4	1.0053	19.4	1.0053	3.10
32	19.0	1.0051	—	—	19.4	1.0053	2.60
33	19.1	1.0050	—	—	19.4	1.0050	2.50
34	19.3	1.0050	—	—	—	—	2.20
35	18.6	1.0051	—	—	—	—	1.50

of the bottom water is about two or three times as great as that of the surface water. The highest density through all stations and depth is 1.0175 (ca. 23.9‰, 15°C.) which was observed at St. 12. This is a salinity roughly corresponding to 70% of common sea water.

In short, that the salinity of this lake is influenced by sea water up to the furthest inward region is remarkable as compared with some other brackish lakes where the salinity is limited to the vicinity of the outlet river. So, when the salinity condition above mentioned is taken as showing the normal state, the entire body of this lake is said to be well suited to the brackish fauna.

*Biological conditions of the lake* As stated above the entire mass of this lake is of typical brackish conditions. Accordingly the fauna inhabiting there is marked by typical brackish animals. Pure fresh-water animals



such as *Carassius auratus* GÜNTHER have not been found. This is of interest when compared with the neighbouring Lake Takahoko, in which the fresh-water fauna living in the innermost region gradually transfers to the brackish fauna towards the outlet region.

Among the fishes found in this lake the eel, *Anguilla japonica* T. & S. is of first importance in fishery. Formerly, until some thirty years ago, when the yield had been very great the size of this fish was comparatively small as compared with the recent fishes which now show a considerable depletion. The salmon, *Oncorhynchus keta* (WALBAUM) and *Oncorhynchus masou* (BREVOORT) which are incomers from the sea are found ascending Futamata River in autumn, though not in great numbers. The Chika (*Hypomesus japonicus olidus* (BREVOORT)) a noted salmonid fish artificially propagated in other lakes is rather scarce in this lake. The Bora, *Mugil cephalus* LINNE and the Menada, *Liza manada* TANAKA are both found in abundance. The Ugui, *Leuciscus hakuensis* GÜNTHER and the Suzuki, *Laterlabrax japonicus* (CUVIER & VALENCIENNES) are both the principal inhabitants. The flat fish *Platichthys stellatus* (PALLAS) is also the commonest fish among the main food fish.

Other than the fishes above stated the Crustacea such as the Mokuzugani (*Eriochea japonica* DE HANN), Nukaebi (*Xiphocardina compressa* (DE HAAN)), Ebijako (*Crangon affinis* DE HAAN), and Tobimushi (*Gammarus* sp.) have been found. Regarding the Mollusca the commonest shell called Iwagai (*Libitina japonicum* (PILSBRY)) was found. This shell inhabits the rocky shores of the lake, and is said to have been found in abundance until a few years ago. The bivalves such as Monoaragai (*Limnaea* sp.), Karasugai (*Cristaria plicata* LEACH?), Yamatoshijimi (*Corbicula japonica* PRIME), and Isoshijimi (*Sanguinolaria olivacea* JAY) are frequent.

**Plankton** The collections of plankton were made twice, i. e. on April 5th and on June 17th, 1937, each collection being made at the centre of the lake at different times of the day. An examination of these specimens showed that the plankton of this lake is typically brackish, and that the change of type due to season is highly distinct. The numerical abundance has been found to be no less than the neritic area of ordinary seas.

In early April (collection of 5th) when the temperature of the water ranged between 5.8°-6.6°C., and the specific gravity around 1.008. Plankton community was dominated by *Skeletonema costatum*. As is well known this is one of the commonest species of marine diatoms. Therefore, superficially it is questionable whether this species might be brought from the sea by the tidal stream. But that this may not be so is surmised

from the absence of other marine diatoms which should be found mixed with this species. Consequently the predominance of this species can be considered as the adaptation of this species for brackish condition. Other phytoplankton in addition to *Skeletonema* were *Melosira*, *Amphiprora*, and *Peridinium* which are all proper to brackish water.

Among the zooplankton which were relatively few in number *Synchaeta pectinata* (?), a species of Rotatoria, was very common. Associated with this species *Asplanchna* sp. was found in second abundance. As the larval plankton two kinds of veliger of Gastropoda and swimming larva of Lamellibranchia were also commonly found.

In mid-June (on 17th) the temperature and specific gravity of the surface water were 17.4°C. (18.5°C., bottom) and 1.005 (1.011, bottom) respectively. Accompanied with such altered conditions the plankton also showed a remarkable change. *Skeletonema costatum* which was the dominant species in April disappeared, and the whole community was replaced by the species of zooplankton. The predominance was exhibited by veliger larva which may probably be evolved from the Gastropoda inhabiting the lake. Greater interest was, however, felt in the point that the nauplius and copepodid of *Limnocalanus sinensis* v. *tenellus*, a typical brackish form, appeared in great abundance. As the adult form was also found mixed with these larvae the reproduction of this species might have probably taken place during May. It is of further interest to consider that this species would provide an excellent food for the fry of herring.

Beside the above species such Rotatoria as *Anuraea cochlearis* var. *tecta* and *Notholca* sp. were found in less abundance. Though the phytoplankton was much less in quantity it was characterized by the appearance of *Biddulphia* sp. which seems akin to *B. levis* of marine form.

#### b) Racial Study

As compared with the herring of Europe where the environmental conditions of this fish are much varied, and hence the form and habit also show a wide diversity, the Japanese herring shows but little complexity in this connection. This is probably due to the relatively uniform condition of environment as seen topographically as well as hydrographically. Under such circumstances the sole cause of varying character in herring seems to be the surrounding of brackish lake which affords an isolated environment to the animal. Indeed the herring found in Lake

Hinuma or Lake Obuchi show such distinct features as to be easily discernible from other herrings. Some of these characteristics are very obvious and can be affirmed without scientific measurement. But not to speak by means of measurement each of these characters can be defined very exactly.

1) *Total length of the body (T)*

a) *Obuchi herring* When all specimens were treated as a whole the length of the body (T) ranged between 25.5–35.0 cm, the mean value measuring 29.9 cm (Table). But when two lots were measured separately the first lot (Table 2) was much larger than the second lot. The former ranged between 27.9 cm and 35.0 cm with the mean of 32.7 cm, while the latter ranged between 25.5 cm and 32.8 cm with the mean of 29.5 cm.

As already stated the first lot was collected at the beginning of the season while the 2nd lot was obtained at the middle or end of the season. This fact suggests that the incoming migration of herring is commenced by larger fishes i. e. old fishes, and that with the advance of the season the school is associated with younger fishes. Such trend is a fact generally known in the herring of Hokkaido.

As can be seen from Table 1 the body length of each age group varied in somewhat wide range. But as will be stated later the mean value of each age group showed very reasonable body length.

b) *Asamushi herring* The total length of the Asamushi herring ranged between 24.3–29.4 cm, with the mean length of 27.9 cm (Table 3). As can be seen from Table 3 this school shows uniformity in size. But this uniformity may have no ecological significance as the fishes were caught by drift net, the size of fish being due to the mesh of the net. Therefore it is probable that the school might be associated with smaller fishes but not with larger, because larger ones, if mixed, could have been caught. And consequently we are led to suppose that the specimen may represent the larger fishes of the school of the year.

2) *Dorsal fin length ( $D_s$ )* The index to denote the relative length of dorsal fin was designated as ( $D_s/T$  1000). On Obuchi herring the mean value and the standard deviation of this index have been found to be  $118.7 \pm 5.2$  and  $111.2 \pm 4.6$  in the Asamushi herring. According to FUJITA and KOKUBO (1927) this number varies very distinctly with races and has the tendency of increasing from north to south as will be seen below. In the following figures, 100 was subtracted from each value, for the convenience of comparison.

(1) Karafuto herring	7
(2) Hokkaido herring	9
(3) Asamushi herring	11
(4) Urajio herring	12
(5) Chosen herring	14
(6) Obuchi herring	19
(7) Tashirojima herring	20
(8) Hinuma herring	19

When seen individually the longest dorsal fin of the Obuchi herring was 32 of Fish No. 79. In the Hinuma and Tashirojima herring the highest value is still larger, showing 38 and 39 respectively (FUJITA and KOKUBO, 1927). From a glance at the above figures it can be seen that the length of the dorsal fin of Obuchi shows close approximation to the Hinuma and Tashirojima herring.

From the equation already given the diversity which exists between the Obuchi and Asamuchi herring can be computed as follows. The difference of two means values was found to be 7.5 (118.7-111.2). The probable errors of these mean values were 0.387 and 0.453 respectively. So that the comparison of two variation curves can be expressed as;

$$-7.5 \pm 0.595$$

As the Asamushi herring was taken as standard negative sign was given to 7.5. The above figures show that the difference of mean values is about 12 times as great as the probable error. If the Obuchi herring is compared to the Hinuma herring the relation between the difference of mean and the probable error is found to be  $1.0 \pm 0.64$ .

3) *Lateral length of the head (Lcpl)* The index to express the relative length of head was designated as  $(Lcpl/T) 1.000$ . In the Obuchi herring this value and standard deviation have been found to be  $182 \pm 4.8$ , and  $200 \pm 3.7$  in the Asamushi herring. According to FUJITA and KOKUBO (1927) this value also varies with races and has a tendency to decrease from north to south as will be seen below. In the following figures, 100 has been subtracted from each index, for the convenience of comparison.

(1) Karafuto herring	103
(2) Hokkaido herring	102
(3) Urajio herring	88
(4) Chosen herring	85
(5) Obuchi herring	82
(6) Tashirojima herring	97
(7) Hinuma herring	88

From the above it will be noted that the change of the length of head is quite contrary to the length of dorsal fin which increases towards the south. Moreover the mode of change is not so regular as in that of the dorsal fin. According to the data of FUJITA and KOKUBO (1927) this value somewhat varies with specimens from lot to lot. In the Hokkaido herring it ranged between 95-107, and in the Hinuma herring it ranged between 82-95. As the value of the Asamushi and Obuchi herring was secured from one lot of each the above values (100 and 80) may be included within the variation range of Hokkaido and Hinuma herring. As observed individually the shortest head of Obuchi herring has been the 72 of the fish No. 42. In Hinuma herring and Tashirojima herring the shortest value was 66 and 72 respectively.

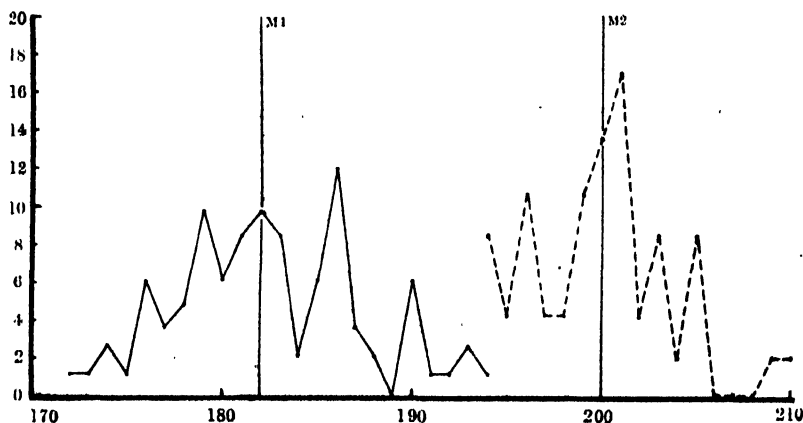


Fig. 3. Comparison of the head-length of Obuchi (—) and Asamushi-herring (---).

Ordinate....frequency in %. Abcissa....head-length ( $\frac{lcp}{l}$ ).

M1....Mean value of the head-length of Obuchi-herring.

M2....Mean value of the head-length of Asamushi-herring.

As in the case of the dorsal fin a comparison of the Obuchi and Asamushi herring were made in relation to variation curve (Fig. 3). The probable errors of the two mean values were 0.357 and 0.396 respectively. The difference of two mean values was found to be 17.5. So that the comparison of two variation curves can be expressed as follows:

$$+17.5 \pm 0.513$$

Above figures show that the difference of mean value is about thirty five times the probable error.

When the Obuchi herring is compared to the Hinuma herring the above relation becomes as  $+6.0 \pm 0.6$ , indicating that this difference is by far smaller than between Obuchi and Asamushi herring.

In short the lateral length of the head of Obuchi herring decidedly differs from that of Asamushi herring. Therefore the distinction indicated by the comparison of dorsal fin length is now strongly emphasized by the measurement of head length.

4) *Body weight (Wt)* The specimens examined ranged between 25.5–35.0 cm in body length, and were relatively few in number. Therefore the weight length relation obtained is naturally limited to the distal part of the so-called weight length curve, and it may be no more than a rough approximation (Fig. 4). In Fig. 4 two lines were added deriving from the data of FUJITA and KOKUBO (1927). These lines can be expressed by the following three formulae which involve the formula  $FL^x = W$ , where  $F$  designates length-weight factor as multiplied by 1000;  $x$ , the power to which  $L$  must be raised in order to express the relation between weight and length;  $W$ , the weight of the fish;  $L$ , the length of the fish.

- (1)  $WL^{3.14}0.0002$ ...Obuchi herring
- (2)  $WL^{3.24}0.0454$ ...Hinuma herring
- (3)  $WL^{3.05}0.6670$ ...Hokkaido herring

From Fig. 5 and the resultant formulae one will note the presence of a marked difference among these three herrings. FUJITA and KOKUBO (1927) emphasized the difference which exists between the Hinuma herring

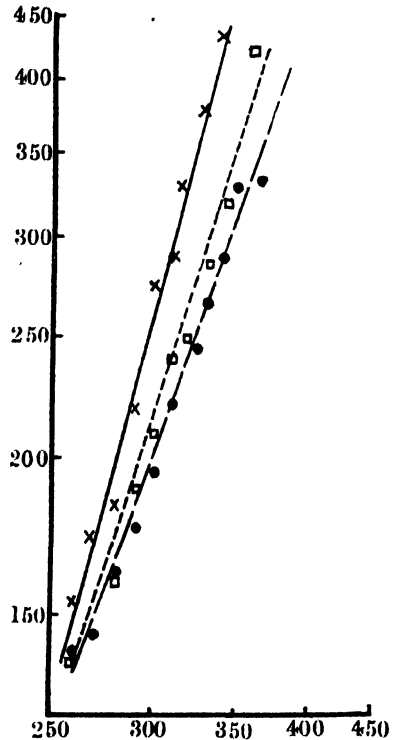


Fig. 4. Weight-length curve for three kinds of herrings.

(x)...Obuchi-herring (□)...Hinuma-herring  
(●)...Hokkaido-herring  
x ● □ are average weight at each cm of length.

Ordinate....Body weight

Abscissa....Body length

and Hokkaido herring. Such a difference is, however, by far exaggerated by Obuchi herring.

5) *Weight of ovary (Owt) and maturity* Preceding the spawning season the weight of the ovary of herring increases. This increase which signifies maturity can be expressed by measuring the *Owt/Wt* i.e. the ratio between the body weight and the ovary weight. FUJITA and KOKUBO's (1927) result shows that in the Hinuma herring the *Owt/Wt* of matured fish of three years (27.5 cm in total length, 120 gms in weight) shows ca. 0.33 i.e. 33%, and ca. 25% in 4 year fish (31 cm in total length, 240 gms in weight). Actually the number of eggs laid increases with the length of the fish, but the ratio *OWt/Wt* decreases with length.

In Obuchi herring the *OWt/Wt* was 12.5% in mean, being 10% or there-about in smaller school (say, 27 cm in length), and over 15% in larger school (30 cm and upwards), including so small a value as 5.2% in the smallest fish (Table 1). None of the fishes showed this value to be over 20%. From these fact it is plain that the schools studied are not yet fully matured, but are on the way to maturity. This can be proved by measuring the *OWt/Wt* of two schools separately. The six fishes exceeding 31 cm, selected from the first lot (taken in December) have the *OWt/Wt* of 10.4%, while the sixteen fishes of same size from the second lot (taken in February) have the *OWt/Wt* of 15%, indicating that the ovary are just growing during this season.

Counting a part of the ovary of a fish it was found that 1 gram of ovary contains 1010 eggs. As the total weight of fishes was actually weighed the calculation from this showed that the mean number of the eggs of a single fish is about 35400.

In Asamushi herring the ratio *OWt/Wt* counted 18.9%, showing that this herring is not yet in ripest condition.

6) *Keel scale ( $K_2$ )* In Obuchi and Asamushi herring the mean number of keel scales and its standard deviation were found to be  $11.40 \pm 0.748$ , and  $10.64 \pm 0.639$  respectively. According to FUJITA and KOKUBO (1927) the mean number of keel scales varies regularly from north to south. In the case of Japanese herring this number ranges between 10.67 and 11.64 as will be seen below:

(1) Asamushi herring	10.64
(2) Karafuto herring	10.67
(3) Hokkaido herring	10.73
(4) Urajio herring	11.14
(5) Chosen herring	11.20

(6) Obuchi herring	11.40
(7) Tashirojima herring	11.45
(c) Hinuma herring	11.64

The number of keel scales in individual herring varies from 6–12 in the former four herrings, and between 10–13 in the latter three. In Karafuto herring and Hokkaido herring the frequency of 10 scaled fish is greater than that of Urajio and Chosen herring, thus decreasing the mean number. In Urajio and Chosen herring the frequency of 11 scaled fish increases resulting in the increase of the mean number. In Tashirojima herring the frequency of 11 and 12 scaled fish equally increases, while the Hinuma herring has the maximum frequency in 12 scale (Fig. 5).

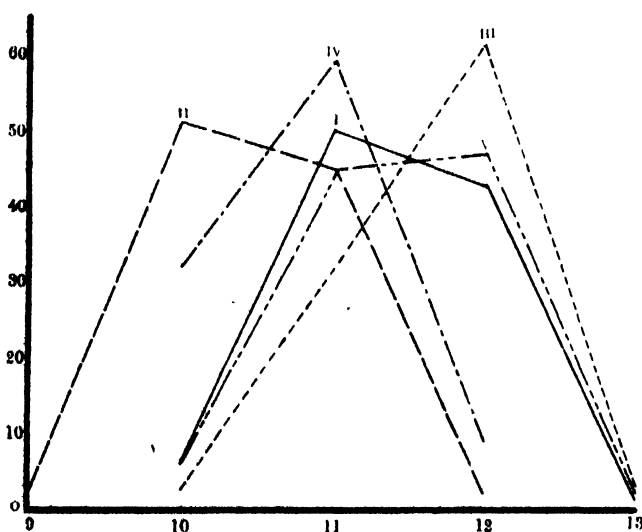


Fig. 5. Comparison of the variation curves of five Japanese herrings.

I....Obuchi-herring                      II....Asamushi-herring  
 III....Hinuma-herring                  IV....Hokkaido-herring  
 V....Tashirojima-herring

Ordinate....frequency in %, Abscissa....number of keel scale.

In this regard, the Obuchi herring markedly approximates to the Tashirojima herring showing almost similar distribution in the frequency of 11 and 12 scales, though the mean number is a little less than the Tashirojima herring. Lake Obuchi is situated between Tashirojima and Hokkaido, so that the number found on Obuchi herring fully agrees with the tendency stated by FUJITA and KOKUBO (1927).



With a view to showing the difference which exists between Obuchi and Asamushi herring a comparison of variation curves was made in respect to the keel scale. The probable error of two mean values of the indexes was computed to be 0.063 and 0.056 respectively. The difference of two mean values was found to be 0.76. So that the comparison of two variation curves can be expressed as follows.

$$-0.76 \pm 0.084$$

The above relation shows that the difference of mean value is about nine times the probable error. Comparison of Obuchi herring to Hinuma herring shows this relation to be  $+0.24 \pm 0.655$ .

7) *Scale* According to LEA (1910) who studied the herring scale very thoroughly the scale taken from the lateral side of the fish (around 6th or 7th row from belly upwards) shows an almost constant character along its entire body length. Therefore, in the present study, a few scales taken from the lateral side around these rows were used for the age determination.

a) *Obuchi herring* On examining the scale we first encountered the difficulty that the scale of Obuchi herring is relatively irregular in its annulus structure. Out of eighty two herrings the specimens which showed normal growth ring were less than half. After close examination the scales were sorted out into three types. The first type to which 48% of the Obuchi herring belonged was characterized by a distinct outermost annulus, and within this annulus somewhat obscure annuli were observed. The second type were such scales as shows more or less typical growth, about 37% of the fishes belonging to this type. The third type had

Age	Percentage	Body length
2	7%	26.1 cm
3	39%	28.0 cm
4	31%	31.3 cm
5	15%	31.8 cm
6	5%	32.8 cm
7	2%	32.8 cm
8	1%	34.0 cm

very indistinct annuli and scarcely enabled the age to be determined, forming about 14% of all specimens (Table 1).

The age determined thus were listed in the following table, from which it can be seen that this school includes the age groups of 2-8 years. The percentage composition of each age group and its

average body length were also given in this table.

From the above it can be seen that this school mainly consists of three and four year fishes, as was just the case with the Hinuma herring (FUJITA and KOKUBO, 1927). The spawning migrants of such an age

composition can be said to be relatively young as compared with the general sea herring, the mixing of 2 year fishes being of further interest.

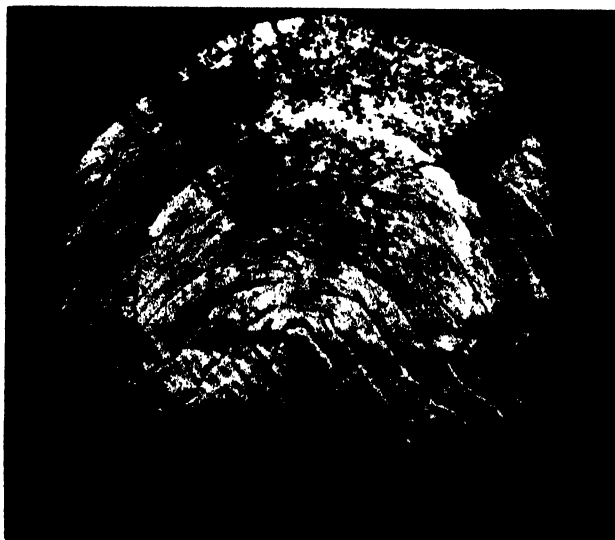


Fig. 6. Scale of two-year fish (Obuchi-herring).

Looking through the ages of the fishes in Table 1 one will notice two salient features. Firstly, the size of 2 year fishes is astonishingly large, attaining 21.0 cm, a size which has not yet been observed in other Japanese herrings. The authors themselves were suspicious at this point at first, but all the scales of these fishes decidedly and obviously indicated their age to be 2 years (Fig. 6). In the second place it is also noteworthy that the size of each age class varies in a relatively wide range, as for instance 3 years ranges between 25.5-31.5, and four years between 27.3-32.7 cm etc. With regard to the size of Japanese herring FUJITA and KOKUBO (1927) reported the superior size of Hinuma herring in contrast to the Hokkaido herring. HJORT (1908) likewise states the varying growth of European herring due to different surroundings. Their statements make the authors consider that the irregular growth of Obuchi herring may be connected with their coastal or brackish surroundings. Indeed, the only way to interpret such growth in herring may account for it as due to a peculiar growth rate proper to such brackish herring.

b) *Asamushi herring* Contrasted with the Obuchi herring the scale of Asamushi herring showed regular and normal growth, so that the age

determinations were made readily (Fig. 7). A few respects which require some comments may be that the growth of the scales of 1 year fishes terminated at the periphery of summer area, thus showing normal growth. While in the scale with 4 annuli the outermost growth zone was relatively narrow as if it had already begun new growth at the periphery of the winter ring.

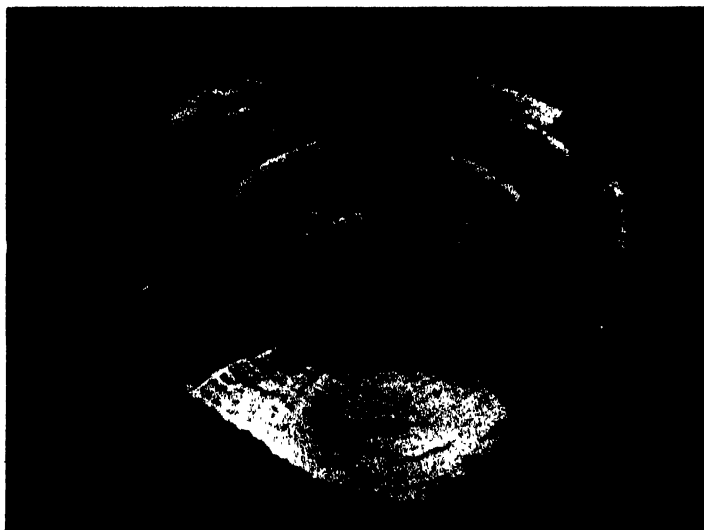


Fig. 7. Scale of four-year fish (Asamusi-herring).

From an examination of the scales it proved that the majority of this school consists of four year fishes mixed with a few of three or five years age. The size of these fishes was of no novelty, being closely allied to that of the Hokkaido herring.

With respect to the study of the scale it has been felt that, in Japanese herring no precise argument can be made in so far as my past and present data are concerned. The question whether it is possible to argue so particularly as LEA (1929) and RUNSTROM (1933, 1936) was reserved for future study.

#### 4) GENERAL REMARKS

##### *a) Herring and surrounding*

Most of the migratory fishes are regulated during the greater part of the year by the supply of food, but during the rest of the year they are under the influence of the spawning desire. Indeed it is during this period

that many neritic fishes such as Salmonidae ascend the rivers to spawn. It seems however, that such a habit has attracted but little attention as regards the herring.

From their study FUJITA and KOKUBO (1927) considered that the anadromous habit of the Hinuma herring may be associated with their racial characteristics. Obtaining the suggestion from them we first intended to study whether their assumption can be realized or not, as we found a similar spawning habit in Obuchi herring. Obuchi and Hinuma herring are so much alike in their habitat and spawning season that one would naturally expect an analogy between them. Both Lake Obuchi and Hinuma communicate with the Pacific Ocean through rivers. The Hinuma river is to the Hinuma herring what the Obuchi river is to the Obuchi herring. But contrary to the above similarity there is an opposing factor in the point that the habitat of Obuchi herring is near that of Hokkaido, where Hokkaido herring abound. This led us to consider the probability that the Obuchi herring might originate from the Hokkaido herring which differs distinctly from the Hinuma herring.

As previously stated, however, the measurement indicated that the Obuchi herring was allied to the Hinuma herring in every particular, differing from the Asamushi herring which shows close approximation to the Hokkaido herring. And hence it may be safe to consider that both the Obuchi and Hinuma herring belong to a race, Pacific Coast Herring, while both Asamushi and Hokkaido herring belong to another race, Japanese Sea Herring. Thus it is likely that the similar surroundings produce similar races.

Speaking of the presence of difference between coastal and sea herring EWART (1883) stated that "If the herrings were at the out set littoral forms some of their descendants would naturally remain in shore throughout the year. And as a result of the different surroundings there might occur some alteration in the form of the body, thus producing two distinct varieties". After stating so he further added "whether this has taken place has not yet been determined". But we think that his assumption was formerly demonstrated by HEINCKE (1874) who distinguished coastal herring from sea herrings. In FUJITA and KOKUBO's (1927) study and also in the present work the form variation which might have been produced by different surroundings is indicated.

In European herring as well as in Japanese herring several marked differences exist between the sea herring and coastal herring. Such differences, in Japanese herring, however, do not coincide with those in

European herring. For instance HEINCKE (1874) indicates that in the European sea herring the number of keel scales is larger and the head is shorter than the coastal herring. But as was stated elsewhere, these relations are completely reversed in the Japanese herring, though the length of the dorsal fin is in both European and Japanese herrings, longer in the coastal than in the sea herring. Therefore it is difficult to determine what character might result from coastal or sea conditions.

b) *Spawning habit*

The spawning of Obuchi herring takes place at the end of March ranging into early April. This has long been known among fishermen, and it has been said that formerly when the herring fishery had not been depleted the milky pollution due to spawning was observed at the spawning ground. Our observation established that there are two spawning grounds, one at the east part and another near the south west part of the lake, the latter being larger than the former. Each of these places measures three or four meters in depth and the bottom is vegetated by hydrophytes.

As previously stated the incoming migration begins from about mid-December, and continues till late March. So that the early emigrants must await spawning for about three months under ice. During this coldest season the reproductive organ which is not ripe at first comes to maturity. This fact strongly suggests the general principle that the reproductive metabolism of organism proceeds under a far lower temperature than that of the vegetative metabolism.

As just mentioned the incoming migration lasts for about three months. Therefore a wide difference of time exists between early and late emigrants. But that this may be also the case with Hokkaido herring is suggested by YAMAGUCHI (1926). He states that the herring shoals which reached the west coast of Hokkaido by early March does not mature as yet. According to him it is over a month later that these herring comes to shore in full maturity. Therefore, in general, it can be said that the herring commences spawning movement very long before maturity. Such a relation of spawning season appears to be also the case in the Pacific herring of North America (JORDAN & EVERMANN, 1934). In San Diego, the southernmost limit of the distribution, they spawn as early as January while in the district further north the spawning takes place much later.

Obuchi herring which are spent and exhausted descend the river during April. The larval fish emerging from their eggs grow to about 5 cm by May or June in the lake and after this the fry descend the river turning into denantant migration. On July 12, 1937 sixty larval fishes (Fig. 8)

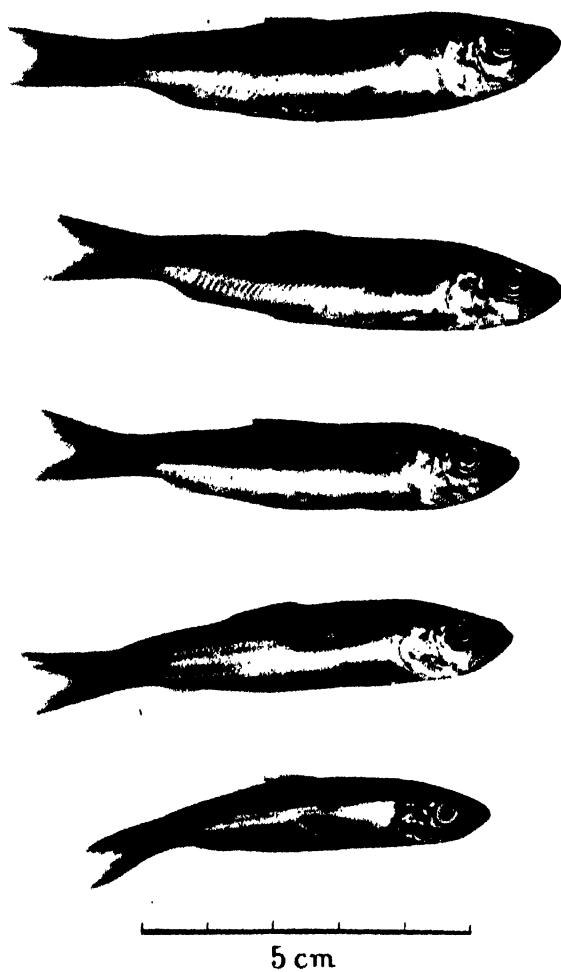


Fig. 8. Larval fishes of Obuchi herring.

were collected at the mouth of Obuchi River, on their way to seaward migration (Table 4). After measuring these fishes it was found that by above season of this year the larval fishes attained to 83 mm in length in maximum, and 65 mm in minimum.

The explanation as to why the Obuchi and Hinuma herring spawn and develop in brackish water may be ascribed to the phylogeny of this fish. Basing on the assumption that the two races of Japanese herring are descendants of one and the same ancestor it can be understood that Obuchi herring might have formed this brackish habit as a result of landward adaptation, as contrasted with Hokkaido herring which has taken the oceanic habit as seaward adaptation. Concerning the susceptibility of the herring to low salinity due to the aforesaid adaptation the authors consider that the extreme of this ability may be 1.005 or upwards in specific gravity. This is not only probable from the study of the Obuchi and Hinuma herring but is also reflected by the fact that in Lake Takahoko, a neighbouring lake of Lake Obuchi, in which the salinity of the central part is 1.003 (TAMURA, 1932. p. 69), but very few herrings are found.

Studying the development of herring YAMAGUCHI (1926) stated that the dilution of salinity seems to have but little effect upon the development even when the salinity was reduced to one third of the sea water. He observed that the larval fish hatched out from the egg survives quite normally under such a low salinity. From his experiment he concluded that the herring develops and survives were it not in the pure fresh water. In this connection FUJITA and KOKUBO (1927) observed that the spermatozoa of Hokkaido herring survives actively for two hours in sea water with a specific gravity of 1.009. These results may prove that in Lake Obuchi the spawning and development of herring would be favourably accomplished.

Regarding the connection of salinity with the development of marine forms HEINCKE (1898) states the capability of salinity to alter and limit the form of marine forms. According to him, in herring, the decrease of salinity is accompanied by a reduction of the number of vertebrae, and an increase in the length of head. FORD (1929) investigated the relation between the salinity of environmental water and the specific gravity of herring larvae. His result suggested that the effect of changing salinity would affect the morphological character of the fish, thus resulting in the racial change.

c) *Racial characters* After studying the races of Japanese herring

FUJITA and KOKUBO (1927) arrived at the conclusion that the characters which best define one race from others are; (1) relative length and height of head, (2) relative length and position of dorsal fin and pelvic fin, this position being expressed as the distance from the tip of snout to the anterior end of fins, (3) number of keel scales between pelvic fin and anus. The relation of these characters in Hokkaido and Hinuma herring, the two distinct races among Japanese herring, is as follows:

	Dorsal fin		Head		Position of pelvic fin	No of Keel scale
	length	position	length	height		
Hokkaido herring	short	far	long	high	far	few
Hinuma herring	long	near	short	low	near	many

In the present study three main characteristics i. e. head length, dorsal fin length, and number of keel scales were selected. From the measurement of these characteristics it was found that Obuchi and Asamushi herring differ widely from each other, and that they respectively belong to the races of Minuma and Hokkaido herring.

HEINCKE (1874) considered the position of the dorsal fin and pelvic fin of the great importance, and distinguished the spring herring of the Baltic from the autumn herring of the North Sea basing on these characteristics. While EWART (1883) compared the position of fins on the spring and autumn herring of Scotland and found that there is even more variation between two spring herrings than HEINCKE finds between his autumn and spring herring. In FUJITA and KOKUBO's (1927) case, however, the position of dorsal fin was one of the most accurate factors in determining the races. In the present study the position of fins has not been measured. But from the correlation which exists between length and position of fins, as is seen in the above table, it is surmised that this relation in the Obuchi herring might be quite reversed as compared with the Asamushi herring.

The significance of counting of keel scale is not only stressed by HEINCKE (1874) but MATHEWS (1883) also attached importance to its counting. In FUJITA and KOKUBO's (1927) study and likewise in the present study it was fully recognised that the counting of keel scales plays an important role in distinguishing races.



## 5) SUMMARY

1) Preliminary observations have been made on the ecological and racial characteristics of the brackish herring found in Lake Obuchi, and on another sea herring collected at Asamushi.

2) General hydrobiological observations have been made on Lake Obuchi, a brackish lake into which the herring invades from the Pacific Ocean.

3) The migration of Obuchi herring into Lake Obuchi begins from mid-December and continues till late March every year. The spawning group of this herring in 1936 chiefly consisted of three and four year old fishes.

4) The spawning of the herring in the lake takes place from late March to early April at two portions of the lake at a depth of three to four meters where hydrophytes vegetate.

5) The spent fish descends the river during late April in an exhausted condition. The larval fishes developed from eggs growing to about 5 cm in length by June when they descend the Obuchi River towards the sea.

6) Asamushi herring migrates to shore probably from Tsugaru Strait during the period from late April to early May. In 1936 the spawning group chiefly consisted of four year old fishes.

7) The salinity of water to which the herring shows normal accommodation seems to be 1.005 or upwards in specific gravity.

8) The racial characters of Obuchi herring are found to coincide with those of Hinuma herring which is a distinct coastal race found by FUJITA and KOKUBO (1927).

9) The racial characteristics of Asamushi herring well coincide with those of Hokkaido herring, and consequently it results in that between Asamushi and Obuchi herring there exists a sharp distinction from racial view point.

10) A gigantic growth of two year fish of Obuchi herring is worthy of note. This point, however, still remains open to question as to whether it may be due to some abnormal growth or to some anomalous structure of the scale.

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TABLE 1.

No.	T			Sex ratio	B.h.	Lcpl	Lcpl T ×1000	Ds	D <sub>s</sub> T ×1000	Wt	O			K <sub>s</sub>	Age	Scale type
	T	T-od	cd								O.Wt	O.Wt Wt (%)	No. of Egg			
1	25.5	22.0	3.5	f.	5.1	4.9	192	3.1	122	149	7.8	5.2	7980	11	3	(III)
2	25.7	22.2	3.5	f.	5.3	4.9	191	3.1	121	154	21.4	18.2	21620	11	2	(II)
3	25.8	22.1	3.7	f.	4.9	4.8	186	3.0	116	140	10.3	7.4	10400	11	2	(I)
4	25.8	22.0	3.8	m.	5.4	4.9	190	3.0	116	152	—	—	—	11	2	(II)
5	25.9	22.3	3.6	m.	5.1	5.0	193	3.1	120	134	—	—	—	11	2	(II)
6	26.0	22.4	3.6	f.	5.3	4.7	181	3.1	119	153	18.7	12.2	18900	11	3	(II)
7	26.3	22.6	3.7	f.	5.6	4.9	181	3.2	122	181	17.4	9.6	17900	12	2	(II)
8	26.3	22.4	3.9	m.	5.3	4.9	186	3.0	114	160	—	—	—	12	3	(II)
9	26.4	22.6	3.8	m.	5.2	4.9	185	3.1	117	168	—	—	—	11	3	(II)
10	26.4	22.3	4.1	m.	5.1	4.8	182	2.9	110	156	—	—	—	12	3	(II)
11	26.4	22.9	3.5	m.	5.4	4.8	182	3.0	114	169	—	—	—	11	3	(II)
12	26.4	22.7	3.7	m.	5.1	4.9	186	2.9	110	152	—	—	—	11	3	(III)
13	26.5	22.8	3.7	f.	5.3	4.9	185	3.0	113	167	18.7	11.2	18900	11	3	(II)
14	26.5	23.2	3.6	m.	5.3	4.9	185	3.1	117	174	—	—	—	13	3	(I)
15	26.6	23.3	4.0	f.	5.3	5.0	188	3.2	120	177	17.0	9.6	17180	12	3	(II)
16	26.7	23.1	3.8	m.	5.4	4.8	180	3.3	124	175	—	—	—	12	3	(I)
17	26.8	23.0	4.1	f.	5.1	5.0	186	3.3	123	160	16.9	10.6	17090	11	3	(I)
18	26.8	23.2	4.0	f.	5.4	5.1	190	3.1	116	173	14.5	8.4	14650	11	2	(II)
19	26.8	23.1	3.6	m.	5.5	5.0	187	3.2	119	177	—	—	—	12	3	(II)
20	26.8	23.4	3.8	f.	5.6	5.0	187	3.0	112	176	20.4	11.5	20310	11	3	(I)
21	26.9	23.3	3.8	m.	5.3	5.0	186	3.2	119	170	—	—	—	11	3	(II)
22	26.9	23.4	3.9	m.	5.7	5.2	193	3.2	119	180	—	—	—	10	3	(III)
23	26.9	24.0	3.7	m.	5.2	5.0	186	3.0	112	181	—	—	—	10	3	(I)
24	27.2	24.4	3.9	f.	5.5	5.0	184	3.1	114	177	12.8	7.2	12990	11	3	(III)
25	27.3	25.6	3.9	f.	5.2	5.3	191	3.1	114	178	18.7	10.5	18900	11	4	(III)
26	27.3	25.9	4.0	f.	5.4	5.2	190	3.4	124	176	22.1	12.5	22390	12	3	(III)
27	27.3	25.9	3.9	f.	5.8	5.2	190	3.4	124	185	18.4	10.0	18900	11	3	(II)
28	27.9	26.2	3.9	f.	5.3	5.3	190	3.2	116	180	13.1	7.3	13220	10	3	(III)
29	28.7	26.1	4.3	m.	5.5	5.2	181	3.3	115	205	—	—	—	12	3	(II)
30	30.0	25.9	4.4	m.	6.2	5.5	183	3.4	113	232	—	—	—	12	3	(I)
31	30.1	25.9	4.4	m.	6.2	5.4	179	3.5	116	257	—	—	—	12	3	(I)
32	30.2	26.2	4.2	m.	6.7	5.3	175	3.5	116	254	—	—	—	12	3	(I)
33	30.2	26.1	4.3	m.	6.2	5.4	179	3.6	119	233	—	—	—	12	4	(II)
34	30.4	25.9	4.2	m.	6.5	5.5	181	3.7	122	236	—	—	—	11	3	(I)
35	30.4	26.2	4.3	m.	5.8	5.5	181	3.7	122	236	—	—	—	12	3	(I)
36	30.5	26.9	4.0	m.	6.2	5.5	180	3.7	121	235	—	—	—	11	4	(I)
37	30.6	26.8	4.4	m.	6.0	5.4	176	3.8	124	235	—	—	—	12	3	(II)
38	30.7	26.9	4.5	m.	6.1	5.6	182	3.6	114	279	—	—	—	11	3	(II)
39	30.7	26.5	4.2	m.	6.8	5.6	182	3.7	121	260	—	—	—	10	4	(I)
40	30.7	26.4	4.3	f.	6.7	5.6	182	3.4	111	212	44.9	24.4	44900	12	4	(I)
41	30.8	26.8	4.0	f.	6.4	5.5	179	3.4	110	263	30.7	14.7	31200	11	3	(I)
42	30.8	26.6	4.2	f.	6.4	5.3	172	3.8	123	237	30.6	13.4	40300	12	3	(III)
43	30.8	26.4	4.4	m.	6.4	5.6	182	3.6	117	239	—	—	—	11	4	(I)
44	30.9	26.6	4.3	f.	6.5	5.5	178	3.6	116	254	43.7	20.7	44300	12	3	(I)
45	31.1	26.8	4.8	f.	6.3	5.7	183	3.5	103	261	43.5	14.5	44600	11	3	(I)

No.	T			Sex ratio	B.h.	LepI	LepI T × 1000	Ds	Ds T × 1000	Wt	O.			K <sub>2</sub>	Age	Scale type
	T	T.cd	cd								O.Wt	O.Wt Wt (%)	No. of Egg			
46	31.1	26.6	4.6	f.	6.7	5.7	183	3.8	122	309	47.1	16.2	47600	12	4	(I)
47	31.1	26.7	4.4	f.	6.2	5.6	180	3.6	116	285	50.0	17.2	56550	12	4	(I)
48	31.2	26.8	4.4	f.	6.4	5.7	183	3.8	122	296	34.6	11.7	34900	11	3	(I)
49	31.2	27.0	4.2	m.	6.5	5.7	183	3.8	122	294	—	—	—	12	5	(I)
50	31.2	26.8	4.6	f.	6.5	5.4	173	3.6	115	320	43.2	14.3	43700	11	3	(II)
51	31.3	26.8	4.5	f.	6.2	5.6	176	3.8	121	292	42.3	14.5	42800	12	4	(II)
52	31.3	26.0	4.3	m.	6.3	5.5	176	3.7	128	296	—	—	—	11	4	(I)
53	31.3	26.7	4.6	m.	6.2	5.6	179	3.9	124	289	—	—	—	12	4	(I)
54	31.4	26.4	4.0	f.	7.0	5.6	178	3.8	121	354	45.9	13.0	46400	11	5	(I)
*55	31.4	26.8	4.6	f.	6.4	5.6	178	3.9	124	285	32.2	11.4	32500	12	4	(II)
56	31.5	27.1	4.4	m.	6.7	5.7	181	3.9	124	307	—	—	—	11	5	(I)
57	31.5	27.0	4.5	f.	6.9	5.9	187	3.8	121	325	59.0	18.2	59800	11	3	(I)
58	31.6	27.1	4.6	m.	6.6	5.5	174	3.9	123	331	—	—	—	12	4	(I)
59	31.6	27.1	4.8	m.	6.8	5.6	177	4.1	130	328	—	—	—	12	4	(II)
60	31.8	27.4	4.1	f.	6.8	5.6	176	3.7	116	338	63.1	18.7	63800	11	4	(I)
*61	31.8	27.1	4.7	f.	6.8	5.7	179	4.0	126	340	30.7	9.1	31000	11	4	(III)
62	31.8	27.3	4.5	m.	6.5	5.6	176	3.6	113	220	—	—	—	11	4	(I)
63	31.9	27.2	4.7	m.	6.1	5.8	182	3.9	122	362	—	—	—	12	5	(I)
64	32.0	27.1	4.9	m.	6.2	5.7	176	3.6	113	299	—	—	—	11	5	(I)
65	32.1	27.4	4.7	f.	6.8	5.8	181	4.0	126	336	44.3	13.2	44800	11	4	(I)
*66	32.1	27.6	4.5	f.	6.5	5.6	174	3.9	122	337	29.4	8.7	29700	12	7	(I)
67	32.2	27.8	4.4	f.	6.5	5.7	177	3.9	121	330	46.7	14.1	47200	11	5	(I)
68	32.2	27.6	4.6	f.	6.5	5.8	180	4.2	131	330	51.5	15.6	52100	12	5	(I)
69	32.2	27.6	4.6	f.	6.3	5.7	177	3.5	109	304	48.8	11.8	49300	11	4	(II)
70	32.3	27.9	4.4	m.	6.8	5.8	179	3.9	121	354	—	—	—	12	4	(II)
71	32.3	27.9	4.4	f.	6.8	5.8	179	3.8	118	326	59.6	18.3	60300	12	5	(I)
72	32.3	27.9	4.4	f.	6.3	6.0	186	4.1	127	314	44.4	16.1	44900	12	4	(II)
73	32.4	27.9	4.5	f.	6.9	5.8	179	3.9	121	360	36.4	10.8	39900	10	4	(II)
74	32.4	27.3	5.1	m.	6.7	5.9	182	3.7	114	339	—	—	—	12	4	(I)
75	32.7	28.1	4.6	m.	6.9	6.0	183	3.6	111	356	—	—	—	10	4	(II)
76	32.8	27.7	5.1	f.	6.6	6.1	186	3.9	119	318	45.5	14.3	46000	11	6	(I)
*77	32.8	28.4	4.4	f.	6.8	6.1	186	3.8	116	373	34.5	9.2	34900	12	6	(II)
*78	32.4	28.4	5.0	m.	7.5	6.2	186	4.1	123	437	—	—	—	12	7	(III)
*79	34.0	29.8	4.2	f.	7.3	6.2	183	4.5	132	482	51.0	11.3	51600	11	6	(I)
*80	34.4	29.1	5.3	m.	7.6	6.4	186	4.0	116	466	—	—	—	11	6	(II)
*81	34.5	29.4	5.1	f.	7.5	6.2	180	3.9	113	447	55.8	12.5	56400	11	5	(I)
*82	35.0	29.9	5.1	m.	7.3	6.5	185	4.3	123	462	—	—	—	11	5	(III)
M.	29.9	25.65	4.25	0.95	6.13	5.45	182.4 ±4.8	3.55	118.7 ±5.2	267.7	35.0	12.5	35400	11.4		

TABLE 2.

No.	T			Sex ratio	B.h.	LepI	LepI T × 1000	Ds	Ds T × 1000	Wt	O.			K <sub>2</sub>	Age	Scale type
	T	T.cd	cd								O.Wt	O.Wt Wt (%)	No. of Egg			
*85	27.9	24.0	3.9	f.	5.2	5.3	180	3.2	115	180	13.1	7.3	13230	10	3	(III)
*86	31.4	26.8	4.6	f.	6.4	5.6	178	3.9	124	285	32.2	11.4	32500	12	4	(II)
*81	31.8	27.1	4.7	f.	6.8	5.7	179	4.0	126	340	30.7	9.1	31000	11	4	(III)
*88	32.1	27.6	4.5	f.	6.5	5.6	174	3.9	122	337	29.4	8.7	29700	12	7	(I)
*77	32.8	28.4	4.4	f.	6.8	6.1	186	3.8	116	373	34.5	9.2	34900	12	6	(II)
*78	32.4	28.4	5.0	m.	7.5	6.2	186	4.1	123	437	—	—	—	12	7	(III)
*79	34.0	29.8	4.2	f.	7.3	6.2	183	4.5	132	482	51.0	11.3	51600	11	6	(I)
*80	34.4	29.1	5.3	m.	7.6	6.4	186	4.0	116	466	—	—	—	11	6	(II)
*81	34.5	29.4	5.1	f.	7.5	6.2	180	3.9	113	447	55.8	12.5	56400	11	5	(I)
*82	35.0	29.9	5.1	m.	7.3	6.5	185	4.3	123	462	—	—	—	11	5	(III)
M.	27.7	23.9	3.7	0.46	5.9	5.9	181.6 ±4.4	3.86	121.0 ±3.6	277.6	26.2	9.9	26800	11.3		

TABLE 3.

No.	T	Sex ratio	Lcpl	$\frac{Lcpl}{T}$ × 1000	Ds	$\frac{Ds}{T}$ × 1000	Wt	O.Wt	$\frac{O.Wt}{Wt}$	No. of Egg	K <sub>2</sub>	Age
1	24.3	m.	5.1	210	2.8	115	140	—	—	—	9	4
2	25.8	m.	5.3	205	2.8	109	144	—	—	—	10	4
3	25.8	f.	5.0	194	3.0	116	151	26.1	17.3	48685	10	4
4	27.0	m.	5.4	200	2.9	107	163	—	—	—	11	4
5	27.1	f.	5.4	199	3.1	114	163	32.5	19.9	60125	10	4
6	27.3	m.	5.6	205	3.1	113	160	—	—	—	11	4
7	27.3	m.	5.4	198	3.1	113	167	—	—	—	11	4
8	27.3	m.	5.3	194	3.1	113	164	—	—	—	11	4
9	27.3	f.	5.5	201	3.0	110	165	28.9	17.5	53465	10	3
10	27.4	f.	5.4	197	3.0	109	152	27.2	17.9	50320	10	4
11	27.6	f.	5.5	199	2.9	105	160	33.0	20.6	61050	11	4
12	27.6	m.	5.6	203	3.2	116	162	—	—	—	10	4
13	27.6	f.	5.5	199	3.0	109	156	26.4	16.9	49840	11	4
14	27.6	m.	5.6	203	3.2	116	163	—	—	—	10	4
15	27.7	f.	5.5	198	3.2	115	188	32.2	17.1	59570	11	4
16	27.7	m.	5.4	195	3.4	123	179	—	—	—	10	3
17	27.7	f.	5.6	202	3.0	108	166	28.7	17.3	53095	10	4
18	27.8	f.	5.7	205	3.3	119	177	31.0	17.5	57350	11	4
19	27.8	f.	5.6	201	2.9	104	170	34.6	20.4	64010	11	4
20	27.8	m.	5.6	201	3.1	111	189	—	—	—	11	3
21	27.8	f.	5.6	201	3.1	113	180	36.4	20.2	67340	10	4
22	27.8	f.	5.7	205	3.0	108	184	24.4	13.3	45140	11	4
23	27.9	f.	5.7	204	3.1	111	180	38.2	21.2	70670	11	4
24	27.9	m.	5.5	197	3.1	111	188	—	—	—	11	4
25	28.0	f.	5.6	200	3.0	107	184	36.8	20.0	68080	11	4
26	28.0	m.	5.6	200	3.2	114	173	—	—	—	10	4
27	28.1	m.	5.7	203	3.1	110	174	—	—	—	10	4
28	28.1	f.	5.6	199	3.1	110	203	41.4	20.4	76590	11	4
29	28.1	f.	5.5	196	3.0	107	190	42.1	22.2	77885	10	4
30	28.1	m.	5.7	203	3.0	107	170	—	—	—	11	4
31	28.2	m.	5.7	202	3.1	110	185	—	—	—	10	4
32	28.2	m.	5.9	209	3.2	113	190	—	—	—	11	4
33	28.3	m.	5.5	194	3.2	113	171	—	—	—	12	5
34	28.3	m.	5.7	201	3.0	106	182	—	—	—	10	4
35	28.4	f.	5.7	200	3.3	116	178	27.7	15.6	51245	10	4
36	28.4	m.	5.7	200	3.3	116	169	—	—	—	10	4
37	28.5	f.	5.7	200	3.2	112	199	35.2	17.7	65120	11	5
38	28.5	f.	5.6	196	3.5	123	210	35.8	17.0	56230	11	4
39	28.5	f.	5.6	196	2.9	102	190	37.2	19.6	68820	10	5
40	28.6	m.	5.6	196	3.1	108	176	—	—	—	10	4
41	28.7	m.	5.6	195	3.0	105	175	—	—	—	11	4
42	28.7	f.	5.7	199	3.4	118	208	39.0	18.8	72150	10	4
43	28.8	m.	5.8	201	3.4	118	193	—	—	—	10	5
44	28.8	m.	5.8	201	3.2	111	185	—	—	—	10	5
45	28.8	m.	5.8	201	3.3	115	201	—	—	—	11	5
46	29.3	f.	5.7	195	3.2	109	190	38.1	20.0	70485	10	5
47	29.4	f.	5.7	194	3.4	116	205	53.8	26.0	98605	10	4
M.	27.86		5.57	199.9 ±2.75	3.11	111.2 ±4.6	175.7	34.3	18.8 ±2.5	62864	10.64	

TABLE 4.

No.	T			B.H.	lcpl		Wt	K <sub>2</sub>
	T	T-cd	cd		lcpl	$\frac{\text{lcpl}}{T} \times 1000$		
1	8.3	7.0	1.3	1.5	2.0	241	4.1	12
2	7.8	6.5	1.3	1.3	1.8	231	3.2	12
3	7.8	6.7	1.1	1.3	1.8	231	3.2	11
4	7.8	6.6	1.2	1.3	1.8	321	3.4	12
5	7.8	6.5	1.3	1.2	1.7	217	3.1	11
6	7.7	6.5	1.2	1.3	1.8	234	3.2	11
7	7.7	6.6	1.1	1.3	1.8	234	3.2	12
8	7.7	6.5	1.2	1.3	1.8	234	3.2	11
9	7.7	6.4	1.3	1.4	1.8	234	3.7	12
10	7.7	6.5	1.2	1.3	1.8	234	3.1	13
11	7.7	6.5	1.2	1.3	1.7	221	2.7	11
12	7.6	6.4	1.2	1.3	1.8	236	3.1	12
13	7.5	6.3	1.2	1.2	1.8	240	2.7	12
14	7.5	6.4	1.1	1.3	1.7	227	3.1	12
15	7.5	6.2	1.3	1.3	1.7	227	3.0	12
16	7.4	6.2	1.2	1.2	1.7	229	2.8	12
17	7.4	6.2	1.2	1.2	1.7	229	2.7	12
18	7.4	6.1	1.3	1.2	1.7	229	2.6	12
19	7.3	6.2	1.1	1.2	1.7	233	2.6	13
20	7.3	6.1	1.2	1.2	1.6	219	2.8	12
21	7.3	6.1	1.2	1.2	1.7	233	2.8	12
22	7.2	6.1	1.1	1.2	1.7	236	2.6	11
23	7.2	6.7	0.5	1.3	1.7	236	2.8	11
24	7.2	6.1	1.1	1.2	1.8	250	2.6	11
25	7.2	6.0	1.2	1.1	1.6	222	2.2	11
26	7.2	5.9	1.3	1.2	1.7	236	2.5	11
27	7.2	6.0	1.2	1.2	1.6	222	2.7	11
28	7.2	6.1	1.1	1.2	1.6	222	2.7	12
29	7.2	6.1	1.1	1.2	1.7	236	2.6	12
30	7.2	6.1	1.1	1.2	1.7	236	2.5	12
31	7.2	6.0	1.2	1.2	1.6	222	2.1	12
32	7.2	6.0	1.2	1.2	1.6	222	2.5	12
33	7.2	6.1	1.1	1.2	1.7	236	2.7	11
34	7.2	6.0	1.2	1.2	1.6	222	3.1	11
35	7.2	6.1	1.1	1.2	1.7	236	2.9	12
36	7.1	6.0	1.1	1.2	1.6	225	2.5	12
37	7.1	5.9	1.2	1.2	1.7	239	2.4	12
38	7.1	5.9	1.2	1.1	1.7	239	2.4	11
39	7.1	5.9	1.2	1.2	1.7	239	2.4	12
40	7.1	5.9	1.2	1.2	1.8	254	2.3	11
41	7.1	6.0	1.1	1.2	1.7	239	2.8	12
42	7.1	5.9	1.2	1.2	1.6	225	2.7	12
43	7.1	6.1	1.0	1.3	1.7	239	2.8	11
44	7.0	5.9	1.1	1.2	1.6	229	2.3	11
45	7.0	6.0	1.0	1.2	1.7	243	2.4	11

No.	T			B.H.	lepl		Wt	K <sub>2</sub>
	T	T-cd	cd		lepl	$\frac{\text{lepl}}{T} \times 1000$		
46	7.9	5.9	1.1	1.2	1.7	243	2.3	11
47	7.0	5.9	1.1	1.1	1.7	243	2.2	11
48	7.0	5.9	1.1	1.2	1.7	243	2.4	12
49	7.0	5.9	1.1	1.2	1.7	243	2.6	11
50	7.0	5.9	1.1	1.1	1.6	229	2.3	11
51	6.9	5.8	1.1	1.1	1.5	217	2.8	12
52	6.9	5.8	1.1	1.1	1.6	232	2.1	11
53	6.9	5.8	1.1	1.1	1.6	232	2.4	11
54	6.9	5.7	1.2	1.1	1.7	246	2.2	11
55	6.9	5.8	1.1	1.1	1.6	232	2.3	12
56	6.9	5.7	1.2	1.1	1.6	232	2.0	11
57	6.9	5.8	1.1	1.3	1.7	246	2.4	12
58	6.7	5.6	1.1	1.1	1.6	239	2.0	12
59	6.7	5.7	1.0	1.1	1.5	224	2.1	12
60	6.5	5.5	1.0	1.2	1.6	246	2.0	12
	7.2	6.1	1.15	1.2	1.7	$\frac{234.6}{\pm 8.37}$	2.7	11.6







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